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A COMPARISON OF THE EFFECTS OF SYMPATHIN AND ADRENINE ON THE IRIS

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In 1921 Cannon and Uridil reported that stimulation of the splanchnic nerves, after removal of the adrenal glands, causes an increased rate of the denervated heart. Since the effect occurred when the portal vein and the inferior vena cava were closed below the liver and did not occur if the hepatic nerves had been cut, the inference was drawn that a cardio-accelerator agent arising in the liver was discharged into the circulating blood. This inference was supported by the observation that when the hepatic nerves were stimulated the arterial blood pressure was elevated—an effect long outlasting the period of stimulation and not explicable as a consequence of a checked blood flow through the liver. The inference was further supported by the experiment of Cannon and Griffith (1922) which proved that blood drawn from the hepatic veins during hepatic nerve stimulation produces cardio-acceleration when injected into the inferior vena cava.

The resemblance between the action of the substance from an hepatic source and the action of adrenine was noted at the time and comparisons were published showing the temporal range of the two agents on blood pressure and heart rate. Years later Rosenblueth and Cannon (1932) demonstrated another similarity of action of these agents in causing contraction of the nictitating membrane.

It is probable that the adrenine-like influence of the substance produced in the liver would early have aroused suspicion of a close relation between it and adrenine but for one reason—it did not cause a dilatation of the pupil. Stewart and Rogoff (1920) had noted that whereas splanchnic stimulation with open adrenal veins evoked a faster heart beat, a rise of blood pressure and an expansion of the iris sensitized by denervation, the same stimulation, after closure of the adrenal veins, though inducing the other changes,

had no effect on the iris. Cannon and Uridil (1921), on stimulating the hepatic nerves, likewise saw the effects on heart and blood pressure but no change in the eye, whereas adrenal secretion affected also the iris.

Since the observations in 1921, evidence has accumulated to prove that what appears in the blood of the hepatic veins when the liver nerves are stimulated is sympathin (cf. Cannon and Rosenblueth, 1933). The close resemblance of sympathin and adrenine has been emphasized (Cannon and Bacq, 1931, and Bacq, 1933a). Bacq (1933b) has reported an expansion of the iris when sympathin is produced by stimulating the lower abdominal sympathetic chains, the hypogastric nerves or the sciatics. Is hepatic sympathin, then, different from that arising in other sources? If so, can the phenomenon be explained? Can the difference of effects of hepatic sympathin and adrenine on the iris be accounted for?

Obviously the organs (heart, arteries and nictitating membrane) which the hepatic substance (whether derived from the smooth muscle or the parenchyma of the liver is not yet determined) affects as adrenine affects them are muscular structures which when they contract are free from the presence of immediate and direct antagonists—at least of their own kind. The iris, on the other hand, with its opposed dilator and constrictor fibers, innervated respectively from sympathetic and parasympathetic sources, presents an almost unique arrangement of autonomic effectors. The present investigation was directed toward learning whether this arrangement might not be significant in accounting for the anomalous actions of sympathin on the iris.

Experiments and results. The observation of Cannon and Uridil (1921) was confirmed, that in the cat under ether anesthesia stimulation of the hepatic nerves caused no change in the iris deprived of its sympathetic nerve supply.

The experimental procedure was then altered. Cats under dial anesthesia were employed. The iris was sensitized by previous removal of the superior cervical ganglion and by injection of cocaine hydrochloride (8 mgm. per kgm.) at the time of test. In order to compare the effects of adrenine and sympathin the contraction of the nictitating membrane in response to sympathin was recorded and then the record was matched as closely as possible by injecting adrenine (commercial adrenalin). In each condition the pupillary changes were measured—in early experiments by means of a millimeter scale fixed in front of the eye, in later experiments by means of uniform photography. As sources of sympathin the liver and the heart were each used; electrodes on the duodenohepatic nerves (with duodenal distribution tied off) and also on the right cardio-accelerator nerves allowed either liver or heart to be tapped at will. Weak stimulation was the rule. No difference between the two sources was observed other than a commonly greater potency and persistence of the action of cardiac sympathin on the iris.

The results of sympathin from the liver or from the heart are shown in table 1 and illustrated in figure 1.

It is clear from table 1 that whereas sympathin, like adrenine, may have the same effect on the denervated nictitating membrane, the two differ markedly in their influence on the iris. An amount of sympathin which matched fairly closely a dose of adrenine in causing contraction of the membrane might produce a relatively slight narrowing or a widening of the pupil or leave it unchanged, whereas adrenine invariably caused a marked dilatation.

TABLE 1

A comparison of the effects of injected adrenine and of sympathin (obtained by stimulating the cardio-accelerator nerves and the hepatic nerves) on the height of the recorded responses (in cm.) of the nictitating membrane and on the responses of the intact iris, expressed as percentage increase (+) or decrease (−) of the transverse dimension before being stimulated

In every animal the effects on the nictitating membrane were matched as closely as possible in order to emphasize the absence of corresponding effects on the iris. The letters A, B, C, etc., designate the different animals employed.

	ADRENINE		CARDIO-ACCELERATORS		HEPATIC NERVES	
	Nictitating membrane	Pupil (per cent change)	Nictitating membrane	Pupil (per cent change)	Nictitating membrane	Pupil (per cent change)
A	2.3	+20	4.4	+10		
	3.6	+30				
B*	1.8	+31	1.7	+40		
	2.0	+50	2.0	+17		
	2.5	+22	3.0	+12		
C	3.0	+67	1.4	−38	4.0	−17
	3.0	+100	1.4	−55		
			4.9	−25		
D	3.8	+100	4.1	+50	6.0	+33
	5.4	+200	4.7	+40		
E	4.5	+110	4.5	+57	4.0	+21
F	2.8	+100	3.0	+32	2.9	−7
G	1.5	+100			1.7	0

* In cat B the pupil dilated continuously throughout the experiment—from 4 to 9 mm.

How might this remarkable discrepancy be explained? Evidently the smooth muscle of the iris was not insensitive—its response to adrenine was quite as delicate as that of the nictitating membrane. And mere presence of antagonistic muscles in the iris offers no clue—adrenine picked out the

dilator fibers and caused them to contract. Of course, quite possibly sympathin was peculiarly ineffective in stimulating the dilator fibers. Or perhaps it stimulated both the constrictors and dilators so that there was more or less of a balance of the antagonistic muscles.

As a means of testing this last idea, cats were anesthetized and the iris was cut radially at one or two places in the circumference, in order to interrupt the circular fibers.[1] The cuts varied in their extent toward the

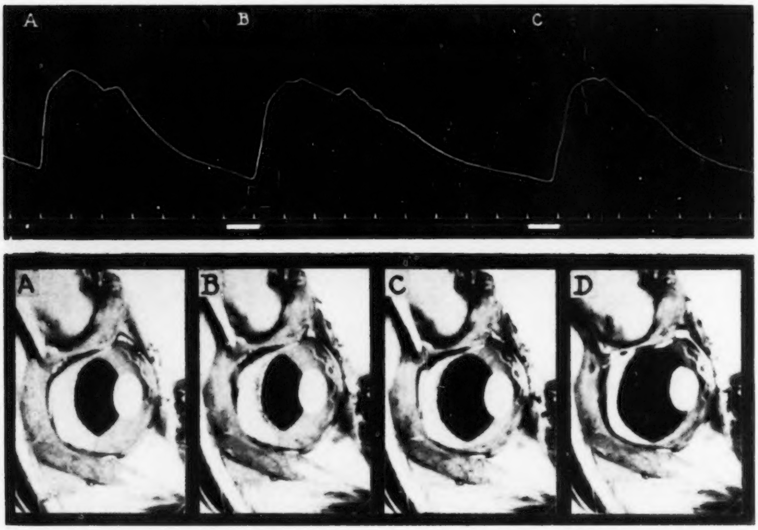

Fig. 1. Experiment F, table 1, October 16, 1934. Dial, cocaine

The graphic records are responses of the nictitating membrane: A, to 0.15 cc. adrenine, 1:200,000; B, to stimulation of hepatic nerves; C, to stimulation of cardio-accelerator nerves. Coil distance, 7.5 cm. Time, half-minutes.

Photographs of the reacting iris: A, control; B, stimulation of hepatic nerves; C, of cardio-accelerator nerves; D, injection of adrenine, 0.15 cc., 1:200,000. The white spot at the right side of the pupil is the corneal reflection of the illuminating bulb.

attachment of the iris. On the side of the operation the superior cervical ganglion was removed. After the aqueous humor had become quite clear (about a month), the influence of hepatic and cardio-accelerator stimulation was tested as before. In table 2 and in figure 2 are shown the results.

It is clear that with only one exception severance of the circular fibers of

[1] We wish to express our thanks to Dr. T. Gundersen for performing some of the operations on the iris.

the iris resulted in a condition which permitted sympathin to dilate the pupil—i.e., caused a contraction of the radiating fibers such as did not occur in the intact organ. To be sure, the expansion was rarely as great as that produced by an amount of adrenine which, by comparative action on the nictitating membrane, could be regarded as equivalent to, or at times less than, the sympathin which was effective. This discrepancy can be explained, we believe, by the fact that usually there was some remnant of contraction in the circular fibers, as shown by presence of the light reflex, in spite of their being cut across.

Discussion. Three explanations of the difference between the action of adrenine and sympathin on the iris might be offered. First, it might be assumed that the two are quite different substances, but that assumption is opposed by the abundant physical, chemical and physiological evidence that they are closely related (cf. Bacq, 1933a). Or second, it might be supposed that the radiating smooth muscle of the iris is impermeable to sympathin, but quite as permeable to adrenine as is the smooth muscle of the nictitating membrane; but that supposition does not account for increased efficacy of sympathin after iridotomy. The third and best interpretation of the different effects of sympathin and adrenine is found, we believe, in the theory of two sorts of sympathin, developed in an earlier research (Cannon and Rosenblueth, 1933).

An explanation of the action of sympathin on the iris. By bringing to bear on the iris the concept that there are two sorts of sympathin, E and I, an explanation of the phenomena, admittedly tentative, can be offered, at least for consideration. In the iris are two sets of smooth muscles, the circular having a parasympathetic innervation, and the radiating, having a sympathetic innervation. The radiating fibers, according to the concept, contain the hypothetical differentiating receptive substance, E, and respond to the combination of adrenine and this substance, i.e., to AE. Also, when the cervical sympathetic nerves are stimulated, they respond to the equivalent of AE, the combination of the chemical mediator of sympathetic impulses, M, and the substance E, or to ME. Consequently they respond to circulating ME discharged into the blood when the heart or the liver is excited by sympathetic impulses.

The circular fibers, subject to parasympathetic nerves, may be assumed to have no E, or none like that in the sympathetic field, and therefore adrenine on reaching them has no defined effect. As a result, adrenine, active only on the radiating fibers, causes dilatation of the pupil. If we suppose, further, that the circulating ME, by bringing the missing E, provides a stimulus which can act on the circular as well as on the radiating fibers, we have a situation which would account for the results reported in table 1; when both sets of fibers are stimulated, sometimes one set, sometimes the other, prevails slightly and sometimes they balance each

other. This relationship becomes especially prominent in the iris because that organ, so far as we are aware, offers the only instance in the body of two smooth muscles acting as direct antagonists.

An explanation of the larger pupillodilator effect of sympathin from the cardio-pulmonary source. Examination of table 1 reveals the fact that although sympathin arising from hepatic nerve stimulation causes little or no dilatation of the pupil, sympathin from cardio-accelerator stimulation (inducing an increased rate of the heart and inhibition of cardiac arteriolar and also bronchiolar muscle) may cause a quite noteworthy widening. In the same category with the latter phenomenon are the observations of Bacq (1933b), previously mentioned, that the pupil dilates when the lower abdominal sympathetic chain is excited—a chain whose impulses cause both contraction and relaxation of smooth muscle. How may the difference between these effects and the absence of such effects on hepatic nerve stimulation be explained?

. The way in which circulating ME alone, from the liver, might act on both circular and radiating fibers of the iris in such a manner as to balance or nearly balance the opposed muscular contractions has been elaborated. When, however, ME and MI are both liberated, from contracted and inhibited muscle respectively, the situation is different. We may suppose that some of the MI will counteract the ME in affecting the circular (constrictor) fibers of the iris, and therefore they would be stimulated less than if ME were alone present. We may suppose further that when MI enters the radiating fibers it dissociates in the presence of E and the M is combined with both I and E, thus becoming effective. But ME also enters the radiating fibers, and, being present there in greater proportion than in the constrictor fibers, the pupil is enlarged. An explanation similar to this was offered to account for the failure of sympathin E to relax the non-pregnant uterus of the cat (see Cannon and Rosenblueth, 1933).

In order to simplify an involved discussion we have assumed thus far that adrenine has no action on the circular muscle. There is, however, evidence which points to an inhibitory effect of the sympathetic and adrenine on the constrictor fibers (Poos, 1927). This relaxation would imply the presence of I, according to the explanation developed above. We must then assume that the E carried by sympathin E is capable of overwhelming the I present, so that contraction ensues. This assumption would lead invariably to larger amounts of ME in the radiating than in the circular muscle. It should not be forgotten, however, that the constrictor is anatomically and mechanically a more efficient muscle than the dilator. Indeed, from this consideration, if equal amounts of ME reached both muscles, constriction should always ensue, which is not the case (table 1). The presence of some I in the constrictor, therefore, explains why dilation sometimes occurs. This presence further offers a clue to the discrepancy

of effects of variable doses of sympathin on the n.m. and the iris, a discrepancy which does not occur with adrenine. Thus in cats C and D (table 1) increasing effects of sympathin on the membrane were not paralleled by increasing effects on the iris, while in cats A and D (table 1) the parallelism of effects with adrenine is patent. If our interpretation of the action of sympathin is correct we should expect, because of the I in the constrictor, that smaller amounts of sympathin E would have a greater dilating effect and less of a constricting action than larger amounts. Table 1 does not contain sufficient data to judge whether this quantitative relationship holds.

TABLE 2

A comparison identical with that in table 1, but with the circular fibers of the iris severed

	ADRENINE		CARDIO-ACCELERATORS		HEPATIC NERVES	
	Nictitating membrane	Pupil (per cent change)	Nictitating membrane	Pupil (per cent change)	Nictitating membrane	Pupil (per cent change)
A	4.4	+43	4.0	+12	4.0	+12
B	6.6	+18	6.5	+18		
C	5.1	+25	6.0	+25	5.7	+25
D	5.7	+21	4.2	+21	5.9	+14
E	8.0	+50	9.5	+25	8.0	0
F	7.4	+50	8.0	+33		

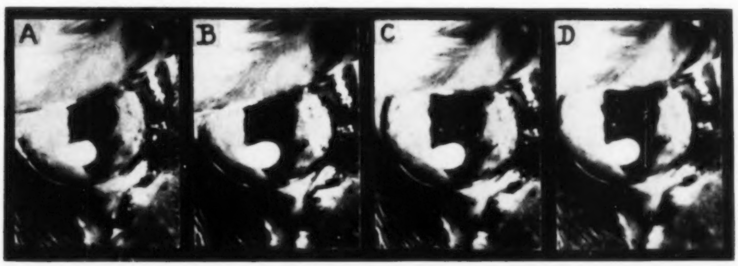

Fig. 2. Experiment C, table 2, October 17, 1934. Photographs of iris with circular fibers severed: A, control; B, stimulation of hepatic nerves; C, of cardio-accelerator nerves; D, injection of 0.1 cc. adrenine, 1:200,000.

SUMMARY

The effects were recorded of the action of adrenine, hepatic sympathin and cardio-pulmonary sympathin on the nictitating membrane and the iris of cats.

When the three stimulating agents evoked nearly equal responses of the membrane adrenine caused a marked dilatation of the pupil, cardiac sym-

pathin caused slight widening, and hepatic sympathin only minor and not consistent effects (table 1 and fig. 1).

Severance of the circular muscle of the iris increased the enlargement of the pupil elicited by sympathin (table 2 and fig. 2).

These results are discussed (p. 255) and the tentative conclusions are reached that sympathin E, unlike adrenine, may affect not only the dilator but also the constrictor muscle of the iris, and that the differences between cardio-pulmonary and hepatic sympathin may be attributed to the presence of sympathin I in the former and its absence from the latter.

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AN ASSAY OF THREE HORMONES PRESENT IN ANTERIOR PITUITARIES OF SEVEN TYPES OF CATTLE CLASSIFIED FOR AGE, SEX AND STAGE OF REPRODUCTION[1]

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We have assayed seven classes of cattle pituitaries for their prolactin, follicle-stimulating (F. S. H.) and thyreotropic content. The three hormones are all present in the late-term embryos examined, in adults of both sexes, in castrate adult males and in pregnant cows. Assays were made on immature male ring doves—each bird indicating, *a*, quantity of prolactin by the crop-gland response (1); *b*, F. S. H. by extent of weight increase of testes (1, 2, 3), and *c*, thyreotropic content by the amount of increase of thyroid weight (4).

MATERIAL AND PROCEDURES. Through essential coöperation of Dr. David Klein of the Wilson Laboratories, Chicago, the heads of various classes of cattle were marked before slaughter at the abattoir of the Wilson Company, the pituitaries removed, placed in proper class, quickly frozen with dry ice and shipped to us. Only a slight thawing of the outermost layer of each package occurred in transit, and thereafter they were kept solidly frozen until prepared for extraction. The posterior lobes were dissected off from all pituitaries except those of the embryos—where small size of the total gland made this impracticable.

The same extraction procedure, yielding only 3 fractions, was used on all of the seven types of pituitaries. After grinding in a meat-chopper the glands were extracted twice at intervals of 2 hours with 6 to 8 times their weight of aqueous-ethanol at a concentration of 60 per cent by volume, and at pH 9.0–9.5 (0.12 cc., n/1 NaOH per gram of fresh tissue). The pH 9.0 insoluble residues are designated hereafter, "A" fractions. The two pH 9.0, 60 per cent ethanol soluble extracts were combined, adjusted to pH 5.0–6.0 and ethanol added to a concentration of 85 per cent by volume. The resulting precipitate, after standing overnight at 0°C., was separated by centrifuging and decanting, and then thoroughly dried with ethanol and acetone; this material contained most of the potency of all

[1] Aided by a grant from the Carnegie Corporation of New York.

of the three hormones and will be called the "B" fraction. The alcohol supernatants at pH 5.0–6.0 are "C" fractions.

It is our experience that the three anterior lobe hormones dealt with here are soluble at pH 9.0 in 60 per cent ethanol and are almost completely insoluble at pH 5.0–6.0 in 85 per cent ethanol. Quantitative assays of all "A" fractions (the initial residues) for their prolactin content showed that those from the pituitaries of bulls and cows in early pregnancy still contained about 30 per cent of the total potency, while the residues of other pituitary types contained less than 25 per cent. Little or no F. S. H. or thyreotropic activity was detected in the "A" fractions at the concentrations injected. The "C" fractions (85 per cent ethanol soluble) from the pituitaries of steers and cows in early and late pregnancy were concentrated to dryness at pH 8.0. An aliquot of each was assayed and an amount of prolactin was found which was equivalent to only 3 to 5 per cent of the total prolactin potency, with only traces of F. S. H. and thyreotropic. Fraction "B," which is soluble in water at pH 8.0, therefore usually contained approximately 70 to 75 per cent of the total prolactin potency and a similar, if not greater, proportion of the other two hormones.

The percentage increase in weights of thyroid and testes (table 1) was obtained from the data on the doves injected with fraction "B." Each of the values given for the "B" fraction is that obtained from six immature ring doves, each dove giving values simultaneously for crop-gland, thyroid and, if a male, testes weights. The same six races of birds were used for assay of the seven pituitary types. The birds were injected once daily for 4 days with 10 mgm. of a particular "B" fraction and killed 96 hours after the first injection. (Ten milligrams of the "B" fraction were equivalent to the number of milligrams of fresh tissue indicated at the bottom of table 1.) Since we do not know at present the exact relationship between dose and response in the case of testes and thyroid enlargement, we are unable to correct our data to the same dosage level in terms of fresh tissue. However, the smallest dosage is 70 per cent of the largest; and at the high level of response obtained here an increase in dosage will not cause a proportional increase of weight; hence the differences in dosage are much less significant than the differences in response.

Prolactin (free of other pituitary hormones) and gonadotropic plus thyreotropic (but free of prolactin), when mixed and assayed on the immature dove, show no synergistic or antagonistic effect upon the crop-gland, testes or thyroid as determined by weight. Pituitrin mixed with these anterior lobe preparations also causes no quantitative change in weight response.

Results. Table 1 gives the potency of the three hormones in the seven pituitary types studied. The units of prolactin given are the *sum* of the units obtained from separate assays of fractions "A," "B," and "C."

Prolactin is present in fairly equal quantity in all pituitary types, excepting only the embryos (mostly 5 to 7 mos.) in which apparently the usual amount per gram of fresh tissue is more than doubled. The pituitaries of cows in late pregnancy (the same cows that provided some of the embryo pituitaries used in this study) had, next to the glands of their embryos, the highest prolactin content. Pituitaries of veal calves and non-pregnant cows have lowest prolactin values, but in the assay of each type an error as great as 20 per cent is probable. Assays of many other batches of freshly frozen unclassified cattle pituitaries have shown that each gram of such fresh anterior lobe contains 30 to 40 units of prolactin. Details of the method of assay of prolactin are given elsewhere (1), and in the

TABLE 1

Prolactin, F. S. H. and thyreotropic hormone content of cattle pituitaries according to age and sex

(Six immature doves used for each test)

HORMONE	MEASURE OF POTENCY	SOURCE OF ANTERIOR PITUITARIES						
		Embryos	Veal calves	Adult steers	Adult bulls	Cows: Not pregnant	Cows: Early pregnancy	Cows: Late pregnancy
Prolactin	Units per gram of fresh tissue*	78+	26	29	34	26	38	44
F. S. H.	Per cent increase of testis weight†	860	975	686	850	837	1215	956
Thyreotropic	Per cent increase of thyroid weight†	63	78	61	93	103	124	82
Fresh tissue equivalent of "B" per day (mgm).		370	525	455	345	500	370	370

* Fractions A+B+C.

† From fraction B only.

present study the racial difference (5) in the crop-gland response is eliminated.

The immature dove testis responds to F. S. H. dosage by increase in weight but no defined quantity of such increase has been identified with a "unit" of F. S. H. (see below). An average testis weight obtained from a control group (8 to 18 birds per group) of ring doves of identical age provided a basis for calculating the amount (per cent) of increase due to F. S. H. injected. Except for a high assay value in the cow during early pregnancy, and a low value for the adult steer (male castrate) the F. S. H. values for the several pituitary types—including the embryos—are approximately the same. Upper limits of rate of increase of testis weight were not attained in the case of any group of males used in these assays.

We are also unable at present to convert a weight increase of the thyroid into terms of "units" of thyreotropic hormone. The results are given in terms of percentage increase relative to the average thyroid weight of birds of the same race. Other tests made by us demonstrate that upper limits of thyroid enlargement were not reached in the present series of assays. It is notable that the pituitary of the bovine embryo is here shown to contain a fairly large quantity of thyreotropic hormone. The two groups of doves used to measure the thyreotropic response from pituitaries of the steer and the bull each contained one bird with a grossly abnormal (large) thyroid which is excluded from our calculation; the values obtained for those two types are therefore perhaps less accurate than other thyroid values.

TABLE 2

Summary of data on total anterior pituitary tissue used, on yield of fraction B and on units prolactin per gland

PITUITARY TYPE	NUMBER OF GLANDS	TOTAL WEIGHT ANTERIOR LOBES	FRACTION B		PROLACTIN	
			Dry weight	Per cent fresh tissue	Total in A+B+C	Per gland
		grams	*grams*		*units*	*units*
Embryos	1,500?	245*	6.5	2.7	19,000	14?
Veal	350	120	2.3	1.9	3,100	9
Steers	200	305	6.6	2.2	9,000	45
Bull	175	262	7.7	2.9	8,800	50
Cow, not pregnant	170	314	6.4	2.0	8,000	47
Cow, early pregnancy	115	252	6.8	2.7	9,600	84
Cow, late pregnancy	140	280	7.7	2.7	12,400	89

* Posterior lobe included in pituitaries of embryos only.

Table 2 supplies additional information on the initial amount of pituitary tissue of each type, on the yield of fraction B, on the total units of prolactin and the prolactin unitage per pituitary.

CONSIDERATION OF RESULTS AND OF PREVIOUS WORK. No quantitative assays of prolactin in the types of pituitary considered here have been previously reported. We have earlier found that cattle pituitaries are a better source of prolactin than are those of sheep or swine (1). The present results demonstrate that all of our seven types of cattle pituitaries are satisfactory sources of this hormone—though highest yields are obtained from embryos and cows in late pregnancy. The high yield from glands in late pregnancy is perhaps comprehensible in terms of the relation of prolactin to lactation. The presence of extraordinary amounts of prolactin in the fetal hypophysis is the most surprising and interesting result of this study. This result indicates that henceforward it must be borne in mind that, in some mammals at least, the embryo is autogenously

supplied with prolactin, F.S.H. and thyreotropic hormones; and that functional or developmental phenomena—induced in fetus or mother by fetal extirpation (Bradbury, 6; Selye, Collip and Thomson, 7) or by expulsion, or by hypophysectomy of mother—may be influenced by the presence of these hormones, especially prolactin, in the pituitary of the embryo.

We are aware of no previous work touching on comparative assay of thyreotropic hormone in pituitaries of the various age and sex types considered here. Weight increase in the guinea pig thyroid has been proposed by Rowlands and Parkes (8) as a superior method for assay of the thyreotropic hormone. The dove is probably not as good as the guinea pig for thyreotropic assays, but the weight changes in the dove thyroid are sufficiently great to warrant our use of it for such comparative assays as are made here. The low thyreotropic content of the steer pituitaries is in accord with usual reports of a low basal metabolism in castrates.

Many studies have demonstrated differences in gonadal response to treatment with pituitaries classified for age, sex or castrate condition. Much of this literature has been recently reviewed by Clark (9, 10) and little would be gained by repetition here. In much of the previous work, however, assays were made on the basis of weight increase in the rodent ovary. In such cases it is now evident that the "augmentation" phenomenon (Fevold and Hisaw, 11) probably had a great and unrecognized influence and that the true F.S.H. content of the implanted tissue or injected extract is still largely unknown. According to Hill (12) the same criticism applies to assays made by the rabbit ovulation test. Much evidence obtained during several years by us indicates that neither the presence nor the absence of any type of luteinizing factor appreciably affects the weight of the immature dove testes used in the present assays.

A few special items from earlier gonadotropic assays require mention. Contrary to our results are those of Bacon (13), who, using the vaginal smear of immature mice for his assay, reported that the pituitary of the non-pregnant cow contains more F.S.H. than the pituitary of the pregnant cow and that the fetal calf pituitary contains still less per gram. On the other hand, Catchpole and Lyons (14) using the rat ovary as their test object, found that the pituitaries from mares in early pregnancy contained much more gonad-stimulating power than those from mares in late pregnancy and that this increase was coincident with the high gonad-stimulating content of the mares' serum The pituitaries from the fetal horse during late pregnancy contained little potency as did the pituitary of the mother.

Clark (10) and many others have found castration in the rat to increase the gonad-stimulating action of the pituitary. In contrast with these results, usually obtained by the implant method, we find that in cattle the anterior lobe of male castrates has a relatively low content of F.S.H. Our results agree with those very recently obtained by Hill (12) for castrate

rabbit pituitaries of both sexes. Hill also made valuable measurements of the relative potency of male and female pituitaries of many species and found that the sex difference depended upon the species.

SUMMARY

Seven classes of cattle hypophyses were assayed for their content of prolactin, follicle-stimulating and thyreotropic hormones. All three hormones were simultaneously assayed on the same group of immature male doves—increased testis weight showing quantity of F.S.H., increased crop-gland weight showing amount of prolactin and increased thyroid weight showing the thyreotropic content. The response in each of these three organs is independent of the others, and is apparently unaffected by the presence of any other anterior lobe hormone.

A suitable extraction method provided only three fractions for injection and assay—one of which contained 70 per cent or more of the total potency. In the case of prolactin all three fractions obtained from each pituitary type were assayed with fair accuracy.

Thyreotropic hormone was found in fairly equal quantity in the seven pituitary types with exception that low values were obtained from the glands of embryos (5–7 mos.) and adult steers.

F.S.H.—in assays uncomplicated by the "augmentation" phenomenon—was found in greatest amount in glands from cows in early pregnancy and least in adult male castrates (steers).

Per gram of moist tissue nearly equal amounts of prolactin (26–34 units) were found in the anterior lobes of veal calves, adult steers, adult bulls and in non-pregnant cows; larger amounts were present in cows in early pregnancy (38 units) and late pregnancy (44 units); a much larger amount (78+ units) was found in the whole glands of 5 to 7 month embryos.

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THE EXHAUSTIBILITY OF THE SYMPATHIN STORES

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The chemical mediation theory of sympathetic nerve activities, as proposed by Cannon and his school, postulates a ready store of the precursors of sympathin, M and H, at the junction of sympathetic nerve fibers and the effectors innervated by them. Both the local and remote (humoral) responses are functions of the concentrations of the mediator. These concentrations are built up by quanta, each nerve impulse (all or none) from each nerve fiber giving rise to a constant minute quantum of mediator (Rosenblueth, 1932b; Rosenblueth and Rioch, 1933). The store of sympathin precursors would seem to be limited, at least under experimental conditions. Repeated stimulations commonly produce diminishing effects (Newton, Zwemer and Cannon, 1931).

The present investigations were undertaken to study the exhaustibility of the sympathin stores and the possibility of duplication of innervation of bilaterally innervated anatomical structures. If there is such a duplication, partial or complete, exhaustion of the sympathin stores by one splanchnic nerve should reduce appreciably the output obtainable from the other.

Method. Cats, under dial anesthesia (Ciba), were used. The right nictitating membrane (n.m.) served as the test tissue. This was denervated by surgical removal of the right superior cervical ganglion 4 to 20 days before the experiment. Further sensitization of the preparation to sympathin was usually accomplished by cocaine (Rosenblueth and Schlossberg, 1931; Rosenblueth, 1931, 1932a), 8 to 10 mgm. per kgm. body weight. Isotonic contractions of the n.m. were recorded as described by Rosenblueth and Cannon (1932) with a magnification of 20 times. Both adrenals were tied off acutely in all animals. In some cases the hepatic nerves (hns.) were also carefully dissected from the hepatic artery and cut when it was desired to eliminate the effects of stimulation of the liver.

The major splanchnic nerve (spl. n.) on each side was approached dorsally at the angle formed by the lowest rib and the lateral border of the

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sacrospinalis muscle. Care was taken to preserve an intact diaphragm and peritoneum. Shielded electrodes were used. Induced shocks (Harvard inductorium, 6 volts in the primary circuit) at a frequency of 15 per second were employed in all experiments unless otherwise indicated. This frequency is well within the limits of the refractory periods for sympathetic nerve fibers and ganglia (Bishop and Heinbecker, 1930, 1932; and Brown, 1934). The timing was controlled by a vibrating spring in the primary circuit, both make and break shocks being effective. Blood pressure (b.p.) was recorded from the left carotid artery by means of a Hürthle type manometer. All injections were made directly into the exposed femoral vein.

RESULTS. 1. *Repetition of separate responses from brief stimulation.* Sympathin production frequently diminishes progressively with successive brief stimulations of a given splanchnic nerve (30 seconds; tetanizing current), as is indicated by successive n. m. and b. p. responses. This, however, is by no means invariably true. It required only a few experiments, however, to show that this method was entirely inadequate to demonstrate the depletion of sympathin. In good sensitive preparations, consecutive responses may be obtained for periods of 6 hours' duration without exhaustion of the sympathin stores. As many as 46 responses have been obtained before the preparation failed from other causes.

2. *Sympathin exhaustion curves following continuous stimulation of the splanchnic nerves at low frequencies, hepatic nerves intact.* In one group of experiments the r. spl. nerve was stimulated continuously (15 times per sec.) until the response of the sensitized n.m. returned to its resting level. The exhaustion times varied between 30 and 78 minutes, average 55 minutes. Obviously, exhaustion here indicated only that the sympathin overflow into the blood had fallen below the threshold values for the sensitized n.m. The maximal heights of the n.m. responses varied from 26 to 76 mm., average 55 mm. Similarly, in other experiments where the l. spl. was stimulated first, the exhaustion times were from 34 to 110 minutes, average 62 minutes; the maximal heights varied from 50 to 78 mm., average 60 mm. The differences here noted between the responses from the two sides are not significant when the nature of the experiments and the individual cases are considered. These results would indicate that the two major splanchnic nerves have approximately equal distributions to the abdominal viscera, not excluding the liver.

The influence of exhaustion of sympathin from the visceral area innervated by one splanchnic nerve on the quantity of sympathin obtainable from the area supplied by the other was next studied. Regardless of which splanchnic nerve was stimulated first, subsequent stimulation of the other usually, but not invariably, gave appreciably inferior n.m. responses. This at first was believed to indicate a duplication of innervation from

the two sides, a belief which later became doubtful in the light of other evidence. In a typical experiment (1), the n.m. response from stimulation of the r. spl. was 76 mm. high and 40 minutes in duration. The general curves for both the n.m. and b.p. responses may be seen from table 1. Similarly, the subsequent n.m. response from the l. spl. stimulation was only 43 mm. high but was 39 minutes in duration. The maximal b.p. rise, however, was 147 mm. Hg as compared with 145 mm. Hg for the r. spl. stimulation. The local production of sympathin, as indicated by the b.p., was far from being exhausted at the time the n.m. response (remote or humoral) failed, since there was a definite fall of b.p. at the termination of the stimulation. This same fact was shown by the marked rise of b.p. on subsequent stimulation of the same nerve even when there was insufficient overflow of sympathin to stimulate the n.m. and when the latter was still sensitive to adrenine (adrenalin, Parke-Davis; 0.1 cc. 1:100,000).

TABLE 1

Blood pressure, mm. Hg; n.m. responses, mm.

EXPERIMENT	SPLANCHNIC NERVE	INDICATOR	EXHAUSTION TIME IN MINUTES									5 MINUTES AFTER END OF STIMULATION
			0	3	5	10	15	20	30	40	48	
1	r.	b.p.	95	200	240	200	155	130	120	115		77
	r.	n.m.	0	62	72	76	68	44	7	0		−4
1	l.	b.p.	78	200	225	200	180	155	130	120		93
	l.	n.m.	0	30	43	25	16	12	10	0		−4
3	r.	b.p.	90	230	200	140	115	95	80	75	70	62
	r.	n.m.	0	62	52	33	33	24	12	6	4	0
3	l.	b.p.	60	140	120	85	75	70	70	68	68	60
	l.	n.m.	0	62	55	50	39	33	20	11	0	0

Furthermore, the local sympathin effect (b.p.) was as great for the l. spl. as for the r. spl. stimulation. This is an additional proof that the local precursors of sympathin were not exhausted.

Since there was ample production of sympathin locally, either it did not reach the n.m. or the n.m. had become less sensitive to the mediator. The latter was indicated by the usual loss of n.m. sensitivity to intravenous injections of adrenine. This was approximately proportional to the diminution in the n.m. response following stimulation of the second nerve. Occasionally this response to the second nerve failed entirely.

In a few experiments the exhaustion times, as indicated by the n.m., were the same for both splanchnic nerves. In experiment 3 (table 1) these were 50 and 48 minutes for the r. and l. sides respectively, the maximal heights being the same in each case; namely, 62 mm. The corresponding maximal b.p. responses were 140 and 80 mm. Hg and the b.p. fall at the

termination of the stimulation was 8 mm. Hg in each case. The fact that the rise of b.p. terminates almost simultaneously with the n.m. response is open to several interpretations; first, in highly sensitized preparations both the permeability of the smooth muscle effectors to sympathin and the sensitivity of the n.m. to this mediator are high (Rosenblueth, 1932a); or second, the impulses set up by the stimulation may have failed to reach the effectors; or third, they may have failed to elicit production of sympathin locally. That the impulses were reaching the effectors can hardly be doubted since identical stimulations, in other experiments, were unquestionably effective for periods 2 to 3 times as long, when other indicators were employed.

Although equal responses of the sensitized n.m. to stimulation of the 2 splanchnic nerves were obtained in a relatively few experiments, the present evidence would indicate that it expresses the true relationship. Additional support for this conclusion has been obtained from summation experiments which will be reported separately.

3. *Sympathin production from the splanchnic area, minus the liver.* With the hepatic nerves (hns.) cut, stimulation of the r. spl. nerve produced an overflow of sympathin into the blood, as indicated by the sensitized n.m., for periods varying between 32 and 58 minutes, average 45 minutes. Similarly, when the l. spl. nerve was stimulated first, the periods varied from 24 to 60 minutes, average 38 minutes. Again there is no significant difference between the capacities of the two spl. nerves and their respective effectors to produce sympathin.

In 2 experiments, after stimulation of the r. spl. had given n.m. curves of 40 and 48 minutes' duration respectively, stimulation of the l. spl. gave responses of 30 and 32 minutes respectively. Regardless of which splanchnic nerve was stimulated first, it always gave superior results. This does not necessarily indicate an overlapping or duplication of innervation for the same reasons as given above.

The results are similar whether the hns. are intact or cut. The liver must not only receive approximately equal innervation from the two splanchnic nerves, but it would seem to have little if any duplication of innervation from the two sides.

4. *The liver alone as a source of sympathin.* Continuous stimulation of the peripheral end of the severed hns. (secondary coil at 7 cm.) gave a n.m. response of 56 minutes' duration. This indicates the time required to exhaust the liver sympathin stores to a level which is subthreshold for the sensitized n.m. It is approximately the same as that required to exhaust the entire splanchnic area to the same level when the hns. are intact, but considerably longer than that for the same area with the hns. cut. The total quantity of sympathin and the maximal heights of the responses were greater when the total area was stimulated undisturbed.

5. *The exhaustion of the local precursors of sympathin.* The local production of sympathin, as indicated by the sustained rise of b.p., may not be exhausted after 2 hours' continuous stimulation at a frequency of 15 shocks per second. Upon terminating the stimulation at the end of this time, the b.p. fell 40 mm. Hg. During this interval a total of 108,000 shocks had reached the effectors, producing an equal number of quanta of sympathin for each nerve fiber stimulated (Rosenblueth, 1932b). Neither "stimulation fatigue" nor failure of conduction of the impulse had occurred. Since, however, the b.p. had not been maintained at its maximal height throughout, the quantity of sympathin liberated per nerve fiber and impulse (quantum) had probably diminished. The overflow of sympathin into the blood had fallen below the threshold for the n.m. at the end of 70 minutes.

Orias (1932) stimulated the cervical sympathetic (preganglionically) at a frequency of 10 shocks per second for 1 hour without evidence of fatigue or block of the nerve elements when the n.m. was used as the indicator. Local sympathin production was maintained. In repeating Orias' experiment, a n.m. response was maintained at more than half of its initial maximal height for 3 hours of continuous stimulation, again a total of 108,000 stimuli for each nerve fiber. The apparent exhaustion was due, in part at least, to local "stimulation fatigue" since shifting of the electrodes to a fresh portion of the nerve led to an immediate rise in the response. The recovery, however, never reached the initial level.

The reliability of continuous stimulation of the splanchnic nerves at a frequency of 15 shocks per second was tested further by determining the persistence of secretion from the adrenal medulla (preganglionic fibers only), employing the sensitized n.m. and b.p. as indicators. Exhaustion was far from complete after continuous stimulation for 2 hours and 40 minutes. This would indicate an output of 144,000 quanta each of adrenine (Rosenblueth, 1932b) and of the chemical mediator acetylcholine (Feldberg, *et al.*, 1934) per nerve fiber stimulated. The relative inexhaustibility of both the acetylcholine and adrenine stores was demonstrated.

SUMMARY AND CONCLUSIONS

With the sensitized n.m. and b.p. as indicators, the exhaustion of various sympathin sources and failure of sympathin effects were studied. The sympathin stores are relatively resistant to exhaustion. It is practically impossible to exhaust them by repeated brief stimulations. The probability of sympathin being exhausted by the normal bodily functions is extremely slight.

Exhaustion of sympathin to the threshold level for remote stimulations (n.m.) occurs earlier than that for its local effects (b.p.). The difference diminishes with increased sensitivity of the preparation. Normally, in the living body, this difference is probably large.

There is usually no appreciable difference in the amount of sympathin which can be produced from a single visceral organ, bilaterally innervated, when one or the other splanchnic nerve is stimulated singly. This holds true whether the hns. are cut or intact. Under suitable conditions, exhaustion of the sympathin stores from stimulation of one splanchnic nerve does not affect the output from stimulation of the other.

The evidence suggests that little or no duplication of innervation by the two splanchnic nerves occurs. The inferior responses frequently obtained from the second nerve stimulations are probably due to secondary factors such as loss of sensitivity of the indicator tissue to sympathin, depreciation of the animal with falling b.p. and inadequate circulation, etc.

Evidence against the loss of functional activity of the nerves involved is given. Blood pressure elevations from stimulation of one splanchnic nerve were observed for 2 hours without failure in any part of the mechanism, while with identical stimulation the adrenal medulla will continue to secrete adrenine for more than 2 hours and 40 minutes without exhaustion. Similarly, the n.m. may be stimulated through the cervical sympathetic nerve for periods of more than 3 hours without exhaustion.

This work was done at the suggestion of Dr. W. B. Cannon. I wish to express appreciation to him for his many helpful suggestions and for the privilege of working in his laboratory.

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DIFFERENTIAL DEPRESSION OF VASOMOTOR MECHANISMS BY ADRENIN

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A peculiar phenomenon in the rat, induced by inactivation of the suprarenal medulla—namely, depressor instead of the usual pressor responses to small doses of adrenalin—was reported by us in 1932 (Wyman and tum Suden, 1932a). These depressor responses were not conditioned by anesthesia, were the result of peripheral vasodilatation (including splanchnic dilatation), and could be induced by removal or ligation of the suprarenal glands. They appeared immediately after such ligation; and were permanent, inasmuch as they were a constant phenomenon in suprarenalectomized rats kept in good health by transplanted cortical tissue.

In spite of this finding in at least one rodent, the rat, the statements are often made that rodents do not possess adrenergic vasodilator mechanisms, and that ergot does not produce in this order of mammals the characteristic reversal of the vasomotor response to adrenalin. Herein is presented a further elucidation of these matters, together with experimental confirmation of our previous explanation of the phenomenon in the rat.

Methods. Carotid blood pressures were recorded by the mercury manometer. Intravenous injections were given in the femoral veins, except in the case of the guinea pig where the external jugular is more accessible. Urethane anesthesia was used, since previous work had shown that it does not interfere with or condition the vasomotor phenomena under consideration. The use of adrenalin chloride (Parke, Davis & Co.) and ergotamine tartrate (1 mgm. per cc.) solutions,[1] was controlled by water or saline injections.

Results. *The effect of ergotamine tartrate.* Although the depressor responses to adrenalin in the rat would obviously be attributed to the stimulation of adrenergic dilator mechanisms, it seems well to verify this point by the use of drugs known to have selective actions on various parts of the vasomotor mechanism, especially in view of the present flux of

[1] The ergotamine tartrate ("Gynergen II") was very kindly supplied by the Sandoz Chemical Works, Inc., New York.

knowledge concerning such problems. Furthermore the effect of ergotamine on vascular responses in the rat appears not to have been reported.

In normal rats (5 expts.) the intravenous dosage of ergotamine necessary to produce a reversal of the pressor responses to injections of adrenalin (from 0.05 cc. of 1:1,000,000 to 0.1 cc. of 1:100,000) was high, at least 1 mgm. per 100 grams body weight. One milligram in a 200 gram rat or 2 mgm. in a 370 gram rat decreased but did not reverse the pressor response. This is in keeping with the well known tolerance of the rat for most drugs and toxins. A typical and complete reversal was obtained, however, with sufficient dosage (fig. 1). It appeared as early as five minutes after the administration of ergotamine in some experiments and persisted for as long as 150 minutes. The effect of the ergotamine itself was a brief preliminary fall in blood pressure followed by a long lasting rise (one or two hours) of about 15 to 30 mm. Hg. That the effect on the responses to adrenalin was not due to the rise in the level of blood pressure was shown by the persistence of the effect in certain cases after the blood pressure had returned to its previous level.

In two rats suprarenalectomized two months previously and kept in good health by autoplastic transplants of cortical tissue the depressor responses to small doses of adrenalin (0.05 cc. of 1:2,000,000 to 0.1 cc. of 1:1,000,000) were doubled in magnitude from 10 to 12 minutes after the injection of 1 mgm. of ergotamine per 100 grams, and tripled some time later (fig. 2). The response to the ergotamine and the reversal of the pressor effects of large doses of adrenalin were the same as in normal rats. Inasmuch as the vascular response to a small dose of adrenalin must be the resultant of its vasoconstrictor and vasodepressor actions, the increase of an already existing depressor response is to be expected after the vasoconstrictors have been paralyzed by ergotamine.

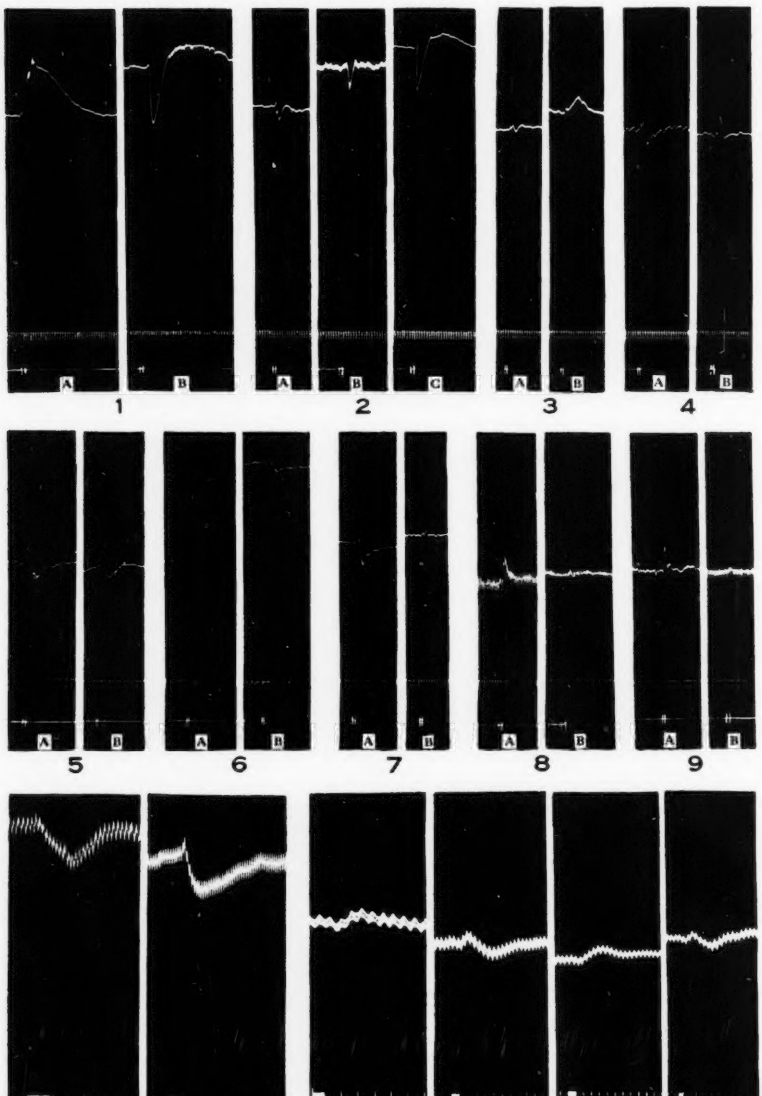
The effect of cocaine hydrochloride. In a normal rat 1 mgm. of cocaine per 100 grams' body weight produced the characteristic sensitization to the pressor action of adrenalin (fig. 3), tripling the pressor responses to small doses (0.05 to 0.1 cc. of 1:2,000,000) and increasing by 16 per cent the response to a large dose (0.05 cc. of 1:100,000). The effect of the cocaine itself was a temporary rise in blood pressure.

In three rats suprarenalectomized from two to eight months previously and having transplanted or accessory cortical tissue small doses of adrenalin (0.05 cc. of 1:2,000,000 to 0.1 cc. of 1:1,000,000) caused depressor or pressor-depressor responses. After the intravenous injection of 0.5 or 1.0 mgm. of cocaine per 100 grams, the depressor responses were reversed and the pressor-depressor responses were converted into pure pressor reactions (fig. 4). Again, sensitization of the vasoconstrictor component of a resultant action would be expected to produce such an effect.

The effects of atropine sulphate and of physostigmine salicylate. In order to determine if cholinergic mechanisms play any part in the depressor responses to small doses of adrenalin in the suprarenalectomized rat, atropine which paralyses such mechanisms and physostigmine which sensitizes them were employed. After doses of atropine sufficient to abolish the effect of faradic stimulation of the vagus nerve (0.1 mgm. per 100 gms.), and of physostigmine sufficient to produce a sustained rise of blood pressure of 10 or 12 mm. Hg (0.01 or 0.02 mgm. per 100 gms.), the minimal pressor responses to small doses of adrenalin in normal rats were not altered, and the responses to large doses showed only such alteration as is characteristic after vagus paralysis by atropine or arterial muscular constriction by physostigmine. In suprarenalectomized rats the depressor responses to small doses of adrenalin were also unaltered after the administration of either drug (figs. 5 and 6). It may be concluded that these depressor responses are due only to the operation of adrenergic mechanisms.

The influence of subcutaneously injected adrenalin. In the previous paper (Wyman and tum Suden, 1932a) the reversal from pressor to depressor of the responses to small doses of adrenalin in the rat, following inactivation of the suprarenal medulla, was interpreted as a manifestation of the release of vasodilator mechanisms, the irritability of which is constantly depressed in the normal rat by a secretion of adrenin induced by the experimental procedure. Hoskins and Rowley (1915) had found that intravenous infusion of adrenalin decreases the irritability of both the pressor and the depressor mechanisms of the dog. A differential depression of the two mechanisms would account for the reversal phenomenon. We had noticed frequently that the depressor responses in suprarenalectomized rats were decreased if the small doses of adrenalin were given very soon after a series of large doses. Conversely, near the end of a long experiment on a normal rat, during which there had been a slow hemorrhage, and when the activity of the suprarenal medulla might well have become diminished, depressor responses to small doses of adrenalin appeared. It was decided, therefore, to study the effect of adrenalin administered in such a way as to produce a slow and constant absorption of small amounts. The small vascular capacity of the rat hinders intravenous infusion, but the same end may be attained by subcutaneous injection of adrenalin (see Wyman and tum Suden, 1932b, p. 292).

In three rats, suprarenalectomized from two to eight months previously and having accessory cortical tissue, depressor responses to small doses of adrenalin (0.05 or 0.1 cc. of 1:1,000,000 or 1:2,000,000) were regularly obtained. Subcutaneous injections of from 0.02 to 0.04 mgm. of adrenalin per 100 grams were given. From one to five minutes after such injections the depressor responses to small intravenous doses of adrenalin were



Figs. 1–11

decreased, and in from three to seven minutes they were completely reversed to pressor responses similar to those obtained in normal rats, and remained so reversed for one or two hours (fig. 7). This strongly supports the interpretation given at the beginning of this section, and also the conclusion that in the rat adrenergic dilator mechanisms are present but that under experimental conditions their action is masked in the normal animal by its own adrenin secretion.

Depressor mechanisms in the cat. Depressor responses to small doses of adrenalin in the cat are well known and have been widely investigated. Accentuation of such responses following inactivation of the suprarenal medulla, however, has not been reported. There recently turned up in the laboratory course a cat which showed unusually pronounced depressor responses to stimuli which ordinarily produce pressor responses in this animal. Under the customary deep urethane anesthesia, strong faradic stimulation of the central end of the femoral nerve (coil at 4 cm.), or intravenous injections of 0.2 cc. of adrenalin, 1:20,000, and of 0.5 cc. of

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Fig. 1. Normal male rat, 200 grams. In this, and in subsequent tracings, the time record shows five-second intervals and is at 0 blood pressure. The lower record signals intravenous injections of adrenalin. Adrenalin, 0.1 cc. of 1:100,000, (A) before and (B) after ergotamine, 1 mgm. per 100 grams.

Fig. 2. Female rat, 165 grams, 49 days after suprarenalectomy and transplantation of cortex. Adrenalin, 0.1 cc. of 1:2,000,000, (A) before, (B) 15 minutes after and (C) 37 minutes after ergotamine, 1 mgm. per 100 grams.

Fig. 3. Normal female rat, 205 grams. Adrenalin, 0.1 cc. of 1:2,000,000, (A) before and (B) after cocaine, 1 mgm. per 100 grams.

Fig. 4. Female rat, 165 grams, 45 days after suprarenalectomy and transplantation. Adrenalin, 0.1 cc. of 1:2,000,000, (A) before and (B) after cocaine, 1 mgm. per 100 grams.

Fig. 5. Female rat, 180 grams, 60 days after suprarenalectomy and transplantation. Adrenalin, 0.05 cc. of 1:1,000,000, (A) before and (B) after atropine, 0.1 mgm. per 100 grams.

Fig. 6. Female rat, 190 grams, 111 days after suprarenalectomy and transplantation. Adrenalin, 0.05 cc. of 1:1,000,000, (A) before and (B) after physostigmine, 0.01 mgm. per 100 grams.

Fig. 7. Female rat, 250 grams, 8 months after suprarenalectomy and transplantation. Adrenalin, 0.05 cc. of 1:2,000,000, (A) before and (B) 14 minutes after subcutaneous injection of adrenalin, 0.02 mgm. per 100 grams.

Fig. 8. Normal male rabbit, 1.85 kgm. Adrenalin, 0.1 cc. of 1:100,000, (A) before and (B) after ergotamine, 4.0 mgm. per kgm.

Fig 9. Normal male guinea pig, 560 grams. Adrenalin, 0.1 cc. of 1:100,000, (A) before and (B) after ergotamine, 5.0 mgm. per kgm.

Fig. 10. Female cat with apparently inactive suprarenal medulla. (A) Strong faradic stimulation of central end of femoral nerve for 13 seconds. (B) Adrenalin, 0.2 cc. of 1:20,000.

Fig. 11. Normal cat. Adrenalin, 0.2 cc. of 1:500,000, (A) before and (B) after ligation of suprarenals; (C) 20 minutes after beginning intravenous infusion of adrenalin, 1:100,000 at 1 cc. per minute; (D) 39 minutes after stopping infusion.

1:50,000 produced pure depressor responses (fig. 10). One-half cubic centimeter of adrenalin, 1:20,000, caused a slight pressor action followed by a marked depressor action, while 0.5 cc. of 1:10,000 caused a marked pressor action followed by a prolonged depressor effect, without the usual secondary rise due to adrenin secretion. It was suspected, therefore, that these phenomena might be the result of inactivity of the suprarenal medulla.

Exact similarity of the effects of faradic stimulation of each splanchnic nerve before and after ligation of each suprarenal gland, and also similarity of the effects of all doses of adrenalin before and after such ligation confirmed this suspicion. Further work was done, therefore, on a normal cat.

The minimal dose of adrenalin which would produce a pure pressor effect in a cat with vagi cut was found to be 0.2 cc. of 1:500,000 (fig. 11, A). Both suprarenal glands were ligated and five minutes later a similar dose of adrenalin produced an effect with a depressor component, while ten minutes after this the response was completely reversed to a small depressor effect (fig. 11, B). This persisted for 128 minutes, when an intravenous infusion of adrenalin solution, 1:100,000, at 1 cc. per minute, was begun and continued for eleven minutes. A slow rise of blood pressure occurred but not more than 5 mm. Hg. Three minutes after beginning the infusion the amount of adrenalin previously used produced again a pressor response similar to that obtained before the cat's suprarenal glands were ligated (fig. 11, C). This persisted until twenty-six minutes after stopping the infusion, when depressor responses again appeared and progressively increased in magnitude until the end of the experiment one hour later (fig. 11, D). Apparently differential depression of vasomotor mechanisms by adrenin is not peculiar to the rat.

The effect of ergotamine in the rabbit and the guinea pig. The acceptance of the statement that rodents do not possess vasodilator mechanisms sensitive to adrenalin depends largely on the universal failure to demonstrate an adrenalin reversal in the rabbit by ergotoxin. Rothlin (1929), however, stated that "between the rabbit on the one hand and the dog and cat on the other, there are only quantitative differences, and . . . it is also possible to produce vasomotor reversal in the rabbit. This, however, is not the rule, but the exception." He did not, in the paper quoted, present any figures or data to support this statement. Since the reversal is easily demonstrable in at least one rodent, the rat, a few experiments were made with two other rodents, the rabbit and the guinea pig.

Bülbring and Burn (1935) say that 0.5 mgm. of ergotoxine per kgm. is usually enough to reverse the pressor effect of adrenalin in the cat, while in the dog larger doses are necessary, namely, from 1.0 to 5.0 mgm. per kgm. In our experiments ergotamine was given in doses of from 1.0 to 4.0 mgm. per kgm. to rabbits (3 expts.) and 5.0 mgm. per kgm. to guinea

pigs (3 expts.). From three to ten minutes later the pressor effects of small or moderate doses of adrenalin (0.1 cc. of 1:1,000,000 to 0.2 cc. of 1:100,000) were much reduced or eliminated in the rabbits and entirely eliminated in the guinea pigs (figs. 8 and 9). In no case, however, did reversal of the pressor effect occur. Apparently some species difference exists among rodents themselves. This point will be further considered in the discussion.

Discussion. The depression of vasomotor irritability by the constant infusion of adrenalin discovered by Hoskins and Rowley, and the differential depression of the constrictor and dilator mechanisms revealed by our experiments must operate to some extent whenever there is a constant liberation of adrenin from the suprarenal glands. This brings one to the question of the biological significance of this apparently anomalous action of adrenin, and especially to the question of why dilator mechanisms are depressed more than the constrictor mechanisms. In our previous work (Wyman and tum Suden, 1932a) we had determined by direct observation of blood vessels that in the rat dilatation of the splanchnic vessels is involved in the depressor reactions to small doses of adrenalin, revealed by elimination of the suprarenal medulla. Is it not possible that the differential depression by adrenin assists homeostasis? When there is a need for redistribution of blood from the splanchnic area to skeletal muscles anything which favors splanchnic constriction would be of use. The beginning secretion of adrenin in such an emergency might be small enough in amount to produce splanchnic dilatation rather than constriction. At the same time differential depression of the vasodilator mechanisms by the same secretion of adrenin might counteract the tendency to splanchnic dilatation until such time as enough adrenin had been secreted to produce the characteristic splanchnic constriction which sends the blood to the dilated vessels. Thus the homeostasis by adrenin would gain significant support.

The interspecific differences within one order of mammals, the rodents, with respect to the possession or demonstrability of adrenergic vasodilator mechanisms, are puzzling. The rat has such mechanisms but ergotamine has not revealed them in the guinea pig and rabbit. It is known that interspecific differences with respect to the nature and distribution of vasodilators do exist. Bülbring and Burn (1935) have concluded that in the dog there are many cholinergic vasodilator fibres in the sympathetic supply to the muscles, while in the cat sympathetic vasodilator fibres are few and adrenergic in function. Adrenalin, however, still has a vasodilator action in the muscles of the dog.

The failure of ergotamine to reverse the pressor action of adrenalin in the guinea pig and rabbit might be explained in several ways. It may be that these rodents do not have adrenergic dilators in sufficient numbers

to be revealed by this method. On the other hand, it may be that in them the adrenergic vasodilator mechanisms are unusually susceptible to the effect of ergotamine and are equally depressed by this drug along with the vasoconstrictors. Therefore, after ergotamine treatment no vascular responses to adrenalin of any kind are obtained. Woods, Nelson and Nelson (1932) ventured the opinion that ergotamine may depress dilators as well as constrictors.

SUMMARY

1. The pressor response to a small dose of adrenalin in the normal rat is reversed by ergotamine (1 mgm. per 100 gms.), increased by cocaine, and unaltered by atropine or physostigmine.

2. The depressor response to a small dose of adrenalin in the doubly suprarenalectomized rat with transplanted or accessory cortical tissue is increased by ergotamine, reversed by cocaine, and unaltered by atropine or physostigmine. It is concluded that this response is due to the stimulation of adrenergic dilator mechanisms.

3. Following the subcutaneous injection of adrenalin in the doubly suprarenalectomized rat with transplanted or accessory cortical tissue, the depressor response to a small dose of adrenalin is reversed. It is concluded that in the normal animal the action of the adrenergic dilators is masked by a secretion of adrenin, induced by the experimental conditions, which differentially depresses dilator mechanisms.

4. A similar reversal of the response to a small dose of adrenalin. from pressor to depressor, following inactivation of the suprarenal medulla, and subsequent reversal from depressor to pressor following the intravenous infusion of adrenalin were demonstrated in the cat

5. Ergotamine (from 1.0 to 5.0 mgm. per kgm.) eliminated but did not reverse the pressor responses to adrenalin in the guinea pig and in the rabbit.

6. It is suggested that differential depression of vasomotor mechanisms by secreted adrenin may be of significance in homeostasis.

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GOAT'S MILK ANEMIA

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It is definitely established that the anemia produced in rats when restricted to a diet of whole cow's milk can be cured by the addition of iron and copper salts. The further addition of manganese is necessary for normal growth and reproduction.

However, a number of workers have reported recently that the anemia produced in children and in rats by feeding goat's milk cannot be cured through the use of iron and copper salts. This anemia was reported to be of the pernicious type; the blood showed a high color index and thus an abnormally low red cell count. Rouminger, Bomskov, and their co-workers (1), as well as P. György (2) state that this condition can be cured by the addition of liver or liver extact. György found that a yeast extract will also cure the condition, and he links the active principle with Castle's "extrinsic factor." He suggests the use of goat's milk anemia in the standardization of liver extracts for pernicious anemia. Bomskov and co-workers (1) have shown that it is not a question of vitamin A, B_1, or B_4 deficiency. Sothmann (3) working in Rouminger's laboratory also found goat's milk to be more potent in supplementing a Borquin-Sherman diet than cow's milk and that lactoflavin had no beneficial effect in the treatment of the anemia.

Since our experience with goat's milk has been quite different from that reported in the literature, we wish to record in this paper the results obtained in our laboratory.

Experimental. The goats used for the milk supply were fed a ration consisting of one part of grain mixture and one part of silage together with alfalfa hay ad libitum. The grain mixture had the following composition:

45 parts yellow corn
25 parts ground oats
20 parts wheat bran
10 parts linseed oil meal
1 part iodized salt

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

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During the fall and early winter a rather poor quality of alfalfa hay was used. During late winter a change was made to alfalfa hay grown on the University Farm. The milk was brought to the laboratory daily in porcelain pails.

All the rats were made anemic according to the method of Elvehjem and Kemmerer (4). In the first experiments the young were fed cow's milk until they were anemic, but in later experiments the young received nothing but the mother's milk and goat's milk. When distinctly anemic (2–3 gm. hemoglobin per 100 cc. blood) the animals were given the supple-

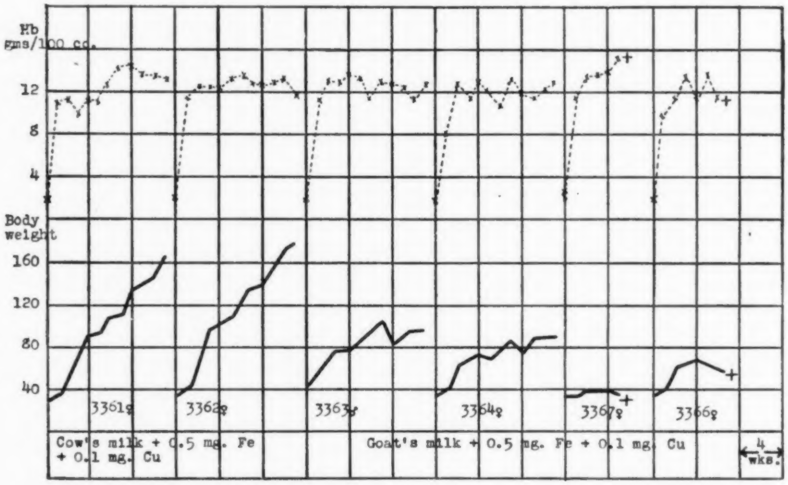

Chart 1. Hemoglobin and weight records showing that hemoglobin regeneration in anemic rats fed on goat's milk plus iron and copper is equally as rapid as in the rats fed on cow's milk plus iron and copper, although the rate of growth on goat's milk is inferior.

ments. Hemoglobin determinations and weight records were made weekly.

Typical results obtained when the rate of hemoglobin regeneration on goat's milk plus 0.5 mgm. Fe as $FeCl_3$ and 0.1 mgm. Cu as $CuSO_4$ was compared to that with cow's milk plus the same supplements are given in chart 1. It is readily seen that all rats showed the same hemoglobin response although the growth of the rats on the goat's milk was much inferior to that obtained with cow's milk. Since these rats were rendered anemic on cow's milk it was possible that certain essential factors might have been stored during the short period when cow's milk was supplied.

In the following experiments the young were never given cow's milk but had access to goat's milk as soon as they consumed food in addition

to the mother's milk. The results for a litter of young reared in this manner are given in chart 2. When severely anemic two rats received the regular addition of iron and copper, two iron alone, and two 0.05 mgm. Mn as $MnCl_2$ in addition to the iron and copper. Those receiving iron alone showed no response and died of anemia, a result similar to that always obtained with cow's milk and iron. All the rats fed both iron and copper showed a very rapid hemoglobin regeneration. The growth again was very poor and one of the manganese rats died very soon after it was started on the experiment but even in this case a very decided increase in hemoglobin had taken place.

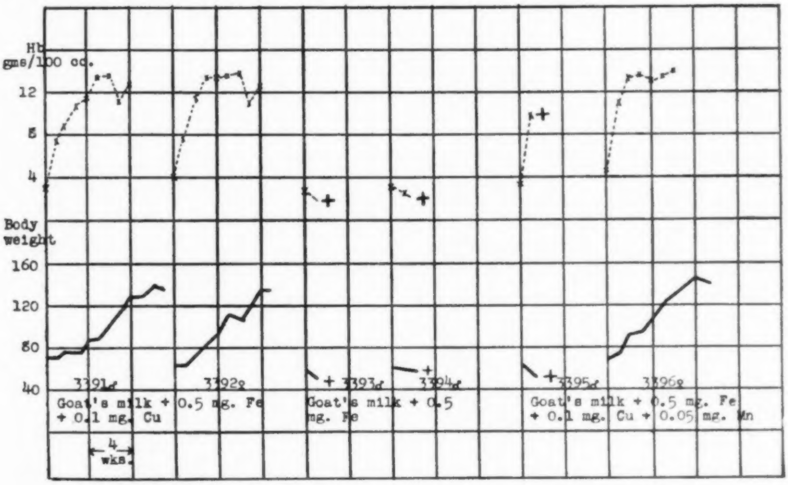

Chart 2. Hemoglobin records and weight curves for anemic rats fed goat's milk plus iron alone, iron plus copper, and iron plus copper and manganese.

Similar results were obtained in a large number of other animals. Erythrocyte counts were made on many of the animals after hemoglobin regeneration and values in the normal range were obtained. However in spite of the rapid improvement in the blood picture, the rats on goat's milk grew very slowly and in many cases stopped growing or even lost weight. These rats showed no definite pathological symptoms, except emaciation and some muscular weakness. Autopsy of the animals which died showed considerable congestion of the intestinal tract. Since the chlorine content of goat's milk is recorded in the literature (5) to be one-tenth of that in cow's milk we thought the condition might be dependent upon a temporary achlorhydria due to lack of sufficient chlorine ingestion. However the addition of 3.3 cc. of 1 N HCl to 100 cc. of milk had no beneficial effect. An analysis of the goat's milk showed it to have practically the same chlorine content as cow's milk.

Some attempt was made to determine the deficiency for normal growth in goat's milk by adding various vitamin concentrates. All the rats were

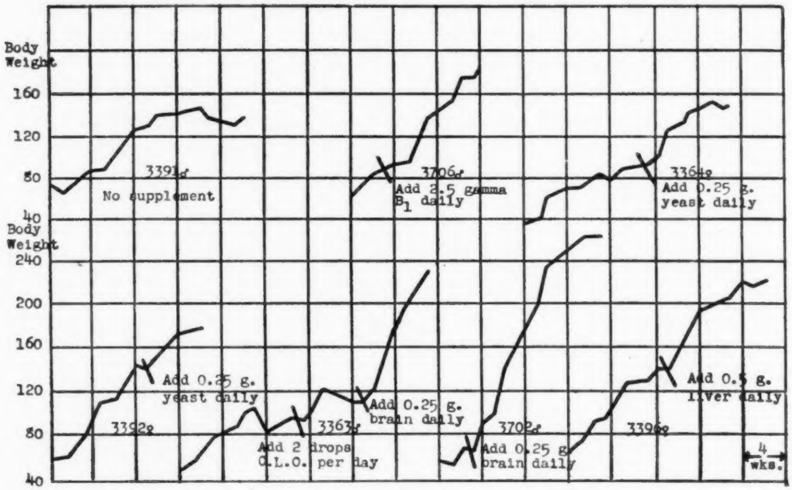

Chart 3. Typical growth curves for rats receiving various supplements to a goat's milk diet. All rats received goat's milk ad libitum, together with 0.5 mgm. iron, 0.1 mgm. Cu, and 0.05 mgm. Mn daily.

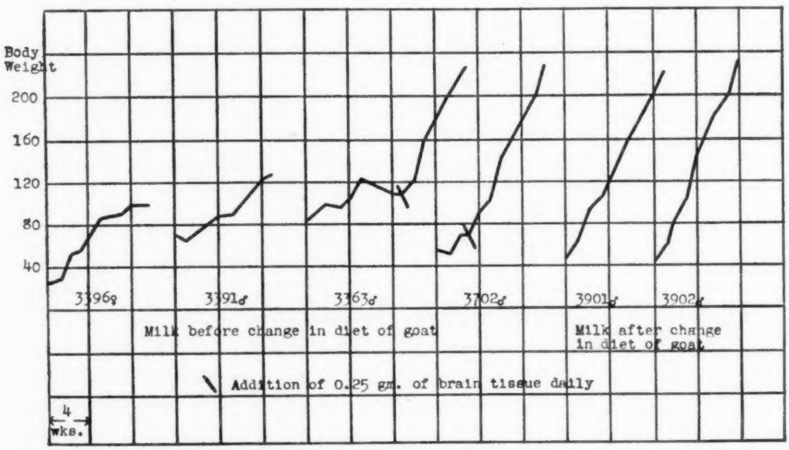

Chart 4. Typical growth curves for rats showing the effect of brain tissue supplement, and of changes in the diet of the goat on growth in rats limited to a diet of the goat's milk plus iron, copper and manganese.

made anemic on goat's milk and then continued on goat's milk with iron, copper and manganese plus the various supplements. Results for addition

of vitamin B_1 crystals (Ohdake[2]), yeast, cod liver oil, liver and brain tissue, are given in chart 3. It is readily seen that vitamin B_1 crystals, yeast or cod liver oil had no distinct beneficial effect. Therefore the deficiency cannot be due to lack of vitamins A, B_1, D, or B_2 (G). Liver produced some improvement but the best results were obtained with brain tissue. Since we have found brain tissue to be a good source of vitamin B_4 it is possible that the goat's milk may have been deficient in this factor. However it is impossible to make a definite conclusion until more experimental work is carried out.

In later experiments when the rats were fed the milk from the same goats receiving a better grade of alfalfa hay much better growth was obtained. Growth records for rats receiving the two types of mineralized goat's milk and the original milk plus brain tissue are given in chart 4. The deficiency for growth observed in goat's milk is dependent therefore upon the diet of the lactating goat. The variations in growth obtained on the mineralized goat's milk are comparable to those obtained by Elvehjem, Hart, Jackson, and Weckel (6) with cow's milk produced at different times of the year.

Discussion. The results presented in this paper demonstrate that goat's milk anemia produced under the conditions in our laboratory is readily cured by iron and copper salts. It is difficult to explain the differences in the results obtained by Bomskov and György and by us. There may, of course, be a difference in the goat's milk used in our laboratory and that used by other workers. However we have observed definite differences in the growth promoting properties of goat's milk without any noticeable change in the response to iron and copper. The rats used in the different laboratories may have differed in the reserve of blood-forming factors, but such variations have never been observed in the case of cow's milk studies. Another possibility is the form of iron salts used. We used a readily available form while both Bomskov and György used either saccharated ferrous carbonate or a basic ferric chloride.

The rapid growth and hemoglobin regeneration obtained with milk produced by goats under certain conditions when supplemented with iron, copper, and manganese shows that goat's milk, like cow's milk, may be deficient in only iron, copper, and manganese. The deficiency for normal growth observed when the goats were on a poor ration indicates that goat's milk varies in nutritive qualities just as cow's milk does. The rate of growth on mineralized milk gives a valuable means of studying the variations in the nutritive value of goat's milk as well as cow's milk until the factor in question is definitely established.

[2] The crystalline B_1 used was kindly donated by Dr. S. Ohdake, Tokyo Imperial University, Tokyo, Japan.

SUMMARY

1. The anemia produced in young rats by restricting them to a diet of whole goat's milk was cured by the addition of iron and copper salts.

2. The growth of rats fed goat's milk mineralized with iron, copper, and manganese was much inferior to that obtained with cow's milk mineralized in the same manner. The deficiency in goat's milk for growth was not corrected by the addition of cod liver oil, yeast, liver, or crystalline vitamin B_1. Normal growth was obtained by the addition of brain tissue.

3. The addition of a better grade of alfalfa hay to the diet of the goats caused a very definite improvement in the growth-promoting properties of the milk.

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A STUDY OF THE MECHANISM OF HYPERTENSION FOLLOWING INTRACISTERNAL KAOLIN INJECTION IN RATS; LEUCOCYTIC REACTION AND EFFECT ON LYMPHATIC ABSORPTION

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Heller (1) and his co-workers have demonstrated the occurrence of peripheral hypertension in dogs following intracisternal injection of suspensions of kaolin and other colloids. This work, as far as we know, has not been repeated in smaller animals, perhaps because of the difficulty in obtaining blood pressure readings. However, in 1934 Griffith (2) reported a method which has proven highly satisfactory in our hands, by which changes in blood pressure can be followed in the intact but anesthetized animal at any interval desired.

This method, in brief, consists of shaving the dorsum of a rat's foot, covering it with cedar oil, and observing the blood flow in the minute vessels by reflected light, using the low power objective of the microscope. A specially fitted blood pressure cuff which is connected to the usual mercury sphygmomanometer encircles the thigh. When the cuff is inflated with a pressure above systolic pressue, flow in the minute vessels stops, to begin again when the pressure is lowered below systolic pressure. We take as the systolic pressure the point at which flow returns. Some experience is required to appreciate the characteristic features of the field and to select suitable vessels, but after a few days of practice there is little difficulty.

Cisternal puncture was done using a hypodermic needle fitted with sealing wax to a glass micropipette. A 30 per cent by volume suspension of kaolin in normal saline was used for injection. The animal under ether anesthesia was pinned to a holder which, with the head flexed, strongly flexed the animal's back in the cisternal region. It was found necessary to have a trough cut out of the holder in the region of the anterior part of the neck so as to avoid pressure on the trachea. Using a clean, but not aseptic, technique the needle was introduced directly into the cistern whereupon the fluid level in the micropipette usually rose. At first we

[1] Henrietta Hecksher Fellow in Medicine.

simply injected the suspension of kaolin (0.02 cc.) at this moment by blowing into the tube. This, however, killed many animals. Later two punctures were made, the first to withdraw fluid and the second to inject. This procedure seemed to be much better tolerated by the animals, presumably because it prevented a sudden increase in intracranial pressure. Blood pressure readings were made at varying times after the injection. The fluid withdrawn before the injection of kaolin was placed on a slide and stained with Wright's stain. At varying times other taps were performed, and the fluid obtained was similarly examined (see below).

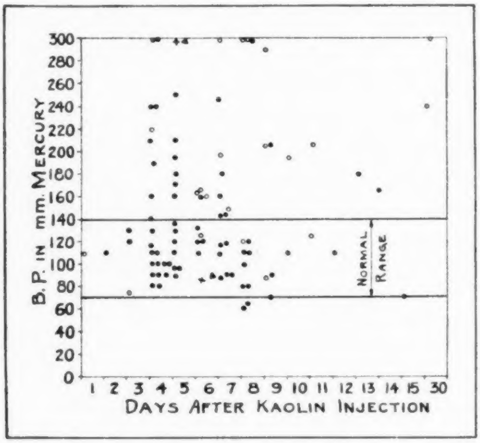

Fig. 1. Chart showing 95 blood pressure determinations on 85 animals made at varying times following the intracisternal injection of kaolin.

○—animals injected with kaolin without previous withdrawal of cerebrospinal fluid.

●—animals in which a preliminary withdrawal of cerebrospinal fluid was made.

△, ×—2 animals in each of which the high reading is probably incorrect (see text).

We have taken the blood pressure in over 300 normal rats, and found that it varies usually from 70 to 130 mm. of mercury, 140 being, we consider, about the upper extreme of the normal range. We have given intracisternal injections of kaolin to 85 rats (see fig. 1), 39 of which developed a hypertension, often quite high. Although the chart shows 95 blood pressure determinations, this represents only 85 animals, as it includes 2 animals which were not hypertensive during the first three days after the injection but were hypertensive after the fifth day, and also a few animals which were given a second injection of kaolin after the first had failed to produce a hypertension. Although 41 animals are listed in the hypertensive zone, we have reason to believe that two of these read-

ings were incorrect (see below). Twelve of these hypertensive animals were used in the present experiment.

The mechanism of the hypertension produced by intracisternal injection of kaolin is not clear. Heller suggested possible irritation of a vasomotor center and presented evidence of increased intracranial pressure, at least for dogs. We are inclined to accept the interpretation that an increased intracranial pressure resulted in our experiments because in repeated taps on such hypertensive animals the cerebrospinal fluid rose more rapidly in the capillary tube and more pressure was required for reinjection. Because of the small amount of cerebrospinal fluid available we made no actual pressure readings.

To determine whether a meningitis developed following the injection of kaolin which might be responsible for the hypertension we examined the cytology of the cerebrospinal fluid. We believed that the presence

TABLE 1

TIME OF CISTERNAL PUNCTURE	NUMBER OF ANIMALS	WHITE BLOOD CELLS PER 2.25 SQ. MM. SMEAR	PERCENTAGE POLYMORPHS	PERCENTAGE MONOCYTES
Normal	17	4		100*
1 day after kaolin	4	436	75	25
2 days after kaolin	3	20	60	40
3 days after kaolin	1	68	55	45
4 days after kaolin	1	2	2	98
5 days after kaolin	4	4	13	87
8 days after kaolin	2	1	8	92
15 days after kaolin	2	2	10	90

* Approximate.

or absence of a leucocytosis in the spinal fluid would be at least suggestive of the existence or nonexistence of a meningeal reaction.

In all 34 spinal fluids were examined, some taken before injection of kaolin and others at various intervals up to 30 days after injection (table 1). Approximately 0.01 cc. of fluid was withdrawn in each instance, placed on a slide, stained with Wright's stain and the white cells occurring in an area of 2.25 sq. mm. counted. While such figures cannot be used to give the number of cells per unit volume of fluid they can be compared relatively. It will be noted that there occurred promptly in the spinal fluid a leucocytosis, consisting largely of polymorphonuclear cells, and that this reached its peak on the first day and after three to five days had returned to normal. It seems unlikely that a meningitis which induced so transient a leucocytosis was responsible for the hypertension, which was relatively permanent.

It seemed possible that kaolin might interfere with the reabsorption of cerebrospinal fluid. Normally probably nearly all of the cerebrospinal fluid is absorbed into the blood stream, but a small portion, perhaps including the colloids, passes along the perineural lymphatics to the adjacent lymph nodes. This latter route appeared to be amenable to study using the method of Mortensen and Sullivan (3).

These workers introduced 2 cc. of thorotrast into the cistern of dogs and 30 minutes later were able to demonstrate the cervical lymph nodes by x-ray. We used a similar technique in 12 normal rats, first taking a control x-ray[2] (see fig. 2, left) then injecting 0.02 cc. of thorotrast intracisternally, and taking subsequent x-rays at intervals varying from 25 minutes to 2 days after injection. We found that thorotrast appeared

Fig. 2. Roentgenogram of neck region of normal rat. Left, before injection. Right, the same animal $2\frac{1}{2}$ hours after the intracisternal injection of thorotrast. The outlines of two nodes show in the submental region, and two more in the area between the trachea and spine.

regularly in the nodes in from 30 to 45 minutes. There was invariably a large distinct shadow of a submental node, and in addition vaguer outlines of nodes were seen in the triangle between the spine, trachea, and mandible (see fig. 2, right). Such nodes when examined microscopically showed a material which corresponded to the published description of thorotrast. As a control it was found that thorotrast injected extracisternally into the deep tissues of the neck did not pass to these nodes.

This same procedure was carried out in 12 rats in which, following the intracisternal injection of kaolin, high systolic blood pressure readings (varying from 180 to 300 mm. of mercury) had been obtained. X-ray examinations were made before injection of thorotrast, and at one and 24

[2] We are indebted to Dr. Alexander Margolies, of the Robinette Foundation, for developing this x-ray technique.

hours after injection. In 10 of these animals no thorotrast appeared in the cervical lymph nodes, and none was seen in the one animal which was x-rayed in addition 10 days after injection. In one of these animals the one hour film showed thorotrast in the ventricles of the brain, though without visualization of the lymph nodes. All 10 of these animals had blood pressures taken at varying periods after the injection of thorotrast, and the persistence of the hypertension was repeatedly confirmed. Of the remaining two animals, one showed a trace of thorotrast in the cervical lymph nodes after 24 hours, and the other showed a more definite shadow in the nodes after the same interval. Both animals were found subsequently to have normal blood pressures. Each had had but a single determination prior to the injection, and this reading had been made with a cuff shown by subsequent studies to be too loose for these animals. To exclude the possibility that mere cisternal puncture without injection of kaolin might impair the passage of thorotrast to the lymph nodes, we injected 3 rats intracisternally with normal saline, waited 4 days, took their blood pressures, which were found to be normal, injected thorotrast in the usual way and obtained perfectly normal lymph node visualization.

As a further control we injected thorotrast by the same technique into a rat made hypertensive (176 mm. of mercury) according to Chanutin's (4) method of subtotal nephrectomy. This animal showed the normal lymph node visualization in one hour following the injection.

We believe, therefore, that kaolin is absorbed along the same perineural lymphatics as is thorotrast, and effectually blocks the passage of the latter. It is to be noted that both our suspension of kaolin and the thorotrast are colloidal preparations. It is suggested that this may be part of the mechanism leading to peripheral hypertension, following the intracisternal injection of kaolin. We conclude that there was no persistent meningeal inflammatory reaction in our experiments. It is possible, however, that the meningeal reaction which gave rise to the transient leucocytosis played a part, either major or minor, in the development of the lymphatic block.

SUMMARY

Thirty-nine out of 85 rats have been rendered hypertensive by intracisternal injections of kaolin. Observations made during repeated cisternal punctures suggested the presence of an increased intracranial pressure, as proven by Heller for dogs, though no actual measurements of cerebrospinal fluid pressure were made in our rats.

Studies of the cytology of the cerebrospinal fluid showed a transient leucocytosis, beginning soon or immediately after injection and subsiding in less than 5 days. The transitory nature of this response was considered evidence against a meningeal inflammatory reaction being the principal factor in maintaining the hypertension, which so far as our evidence to date goes, has been permanent.

The passage of thorotrast from the cistern to the cervical lymph nodes was demonstrated by x-ray. This passage to the lymph nodes did not occur in the animals that had been made hypertensive by the intracisternal injection of kaolin, a fact which suggests that one effect of the kaolin is to interfere with lymphatic absorption.

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BASAL METABOLISM AND URINARY NITROGEN EXCRETION OF ORIENTAL WOMEN

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As possible causes of the low metabolism found with Orientals (1, 2, 3)—causes other than the emphasized racial factor—undernutrition, climate, physical configuration, and particularly the amount of protein in the diet have been frequently suggested. This present investigation was undertaken primarily to determine whether the Oriental's low basal metabolism is associated with a low protein metabolism, specifically a low urinary nitrogen excretion. Five foreign-born Oriental women and, as controls, six Caucasian women served as subjects for the study of both these factors. In addition, basal metabolism measurements unaccompanied by urine studies were made on six other Oriental women. The pertinent data are recorded in tables 1 and 2. The young women were occupied with their regular student duties at Mount Holyoke College. The Orientals had been living in the United States for from one to three years, each lived in the same college dormitory as her control and ate at the same table, and the food served to both was the same. The particular girl acting as control for each Oriental is indicated in table 2, T. W. being the control for M. P. and so on.

The body weights of the Orientals were, with the exception of one of the Japanese, considerably lower than those of the controls. This is not surprising, since many Orientals are of smaller frame than Caucasians. The pelidisi, according to Pirquet (4) an index of the state of nutrition (computed from the calculated sitting heights and the body weights), ranged from 92 to 98 with the Caucasians. With the Orientals they were 83, 88, and 88 in three instances and in the other eight instances from 90 to 95. Those Orientals with pelidisi under 90 (Y. C. M., W. Y. H., and V. A.) may have been somewhat undernourished, but certainly the other eight were as well nourished as the Caucasians.

A closed-circuit respiration apparatus with mouthpiece, valves, and spirometer was used. No observations were made on the first two days of the menstrual period nor on later days, if the subject suffered discom-

TABLE 1

Basal metabolism of Oriental and Caucasian women

SUBJECT	DATE	AGE	WEIGHT	HEIGHT	RESPIRATION RATE	PULSE RATE	O_2 PER MIN.	DEVIATION FROM HARRIS-BENEDICT PREDICTION
	1930	*years*	*kgm.*	*cm.*			*cc.*	*per cent*
Chinese:								
A. Y.	May 28*	20	44.5	154	13	69	159	−13.3
H. H. G.**	May 19*	20	53.0	163	10	56	176	−10.4
Y. C. M.	June 1*	21	44.8	163	12	63	169	−8.4
W. Y. H.†	June 1‡	35	42.2	157		69	156	−8.6
N. L.	May 3	20	47.5	156	13	67	167	−11.1
N. L.	June 3	20	47.5	156	12	73	156	−16.9
C. Y. C.	May 5	24	43.2	152	12	60	147	−17.5
C. Y. C.	June 4	24	43.2	152	15	64	142	−20.5
Japanese:								
F. M.†	June 1‡	25	35.5	148		63	140	−15.5
A. N.	May 7	21	58.6	166	11	59	206	+0.5
A. N.	May 30	21	58.6	166	12	51	204	−0.8
Korean:								
S. K.§	June 6*	24	46.6	157	16	67	163	−11.4
M. P.	April 11	22	40.3	150	10	76	156	−10.8
M. P.	May 28	22	40.3	150	9	67	142	−19.0
South Indian:								
V. A.	May 18	21	37.6	163	17	71	158	−9.9
Caucasian:								
T. W.	April 25	21	54.5	159	16	70	195	−1.1
A. H.	May 9	18	55.6	165	15	67	165	−18.7
H. N.	June 1	19	65.4	167	17	57	212	−2.1
R. P.	May 3	19	51.4	161	14	70	182	−6.6
R. P.	June 5	19	49.8	161	16	65	180	−6.8
L. G.	May 12	19	59.8	171	13	69	205	−1.9
J. C.	May 17	19	63.4	165	15	71	230	+7.9

* 1927.

** Athletic; most vigorous of the Orientals; winner of tennis championship.

† Measured in 1926 by Prof. H. L. Stedman. F. M. was likewise measured in 1924 when her basal metabolism was 9.9 per cent below the Harris-Benedict prediction (1).

‡ 1926.

§ An infantile paralysis victim, but well and strong at time of measurement. The paralysis was in one leg. A recent operation permitted her to walk much more than when she first came to the United States.

fort. The room temperature averaged 23°C., and the mouth temperature varied from 97.3° to 98.4°F. The respiration rates and the pulse rates of the Orientals were not significantly different from the rates noted with the controls, although the respiration rates of the former were in general somewhat lower.

The Orientals who were studied on two days had in each instance a metabolism somewhat lower (on the average 5 per cent) on the second day, although in two cases the pulse rates were higher on the second day. Since none of these subjects had had previous experience with metabolism measurements, the agreement between the first and second days is reasonable. One of the Orientals, A. N., had a metabolism closely approximating the Harris and Benedict prediction (5). She was an unusually large Japanese (no admixture of western blood) who told us that the men in her family on both sides had for thirteen generations been large, that her father liked American food and ways of living, and that consequently many of the food habits in her family had been western. The other Orientals had a metabolism averaging 12 per cent below the prediction standard. In the Caucasian group the metabolism of A. H. was appreciably below the prediction, possibly because (as she claimed) she had been for several months on a low protein diet. The measurements on the other Caucasians were nearer the standard than were those on the Orientals.

The general picture shown by these data is that the Orientals had a low basal metabolism, both compared with the prediction standard and compared with their own particular Caucasian controls. Since the tendency is for the basal metabolism to be lower on subsequent days of measurement than on the first day and since for the most part our observations were confined to one day, the data reported in table 1 represent maximum rather than minimum values. From this standpoint the picture of a low metabolism with the Orientals is all the more striking. Although three of the Orientals had pelidisi below 90, suggesting that they were somewhat undernourished, inspection of our earlier data (1, 2, 3) and of the present series indicates that in general the Oriental's low metabolism is seemingly independent of the state of nutrition. This present research is confirmatory of the low metabolism noted in earlier measurements on Orientals, both at Mount Holyoke College (1) and at Denison House in Boston (2). The latter measurements were made on American-born Chinese girls who had always lived in the United States, whereas the present series and the earlier series at Mount Holyoke College were made on foreign-born Chinese and Japanese girls who were living temporarily in an American college, under American conditions as regards food, clothing, environment, and daily activities. Hence it appears that this tendency for Orientals to have a low metabolism persists under these different conditions.

The urine was collected in 24-hour periods on five days within five to twelve days of each other, between May 3 and May 27, 1930. The total urinary nitrogen excretion per day averaged 8.3 grams with the Caucasians and 7.5 grams with the Orientals. According to the experience of the Mount Holyoke laboratory over a number of years the average Caucasian girl probably has a total nitrogen output in the urine of not far from 10 grams daily.[1] Among our Caucasian controls this amount was exceeded only by L. G. The nitrogen output per kilogram of body weight per 24 hours (see table 2) varied considerably from day to day. A questionably low value was found on the second day with the control A. H., who was on a low protein diet, and yet she maintained that all the urine voided on this day had been collected. With the Caucasian H. N., substituted as a control in place of A. H., one day's collection of urine only was secured,

TABLE 2

Urinary nitrogen excretion per kilogram of body weight per 24 hours

SUBJECT	DAY TO DAY EXCRETION	AVERAGE
	mgm.	*mgm.*
Orientals:		
M. P.	204, 151, 177, 165, 171	174
N. L.	232, 173, 189, 209, 206	202
C. Y. C.	179, 143, 143, 153, 137	151
A. N.	163, 124, 110, 114, 95	121
V. A.	257, 170, 242, 163, 114	189
Caucasians:		
T. W.	140, 150, 104, 143, 143	136
A. H.	127, 64, 135, 152, 181	132
R. P.	147, 148, 192, 168, 159	163
L. G.	211, 200, 157, 228, 169	193
J. C.	136, 116, 89, 99, 81	104

showing a nitrogen excretion of 133 mgm. per kgm. per 24 hours. In three instances the average nitrogen excretion per kilogram of the Orientals was greater than that of their controls and in two instances lower. The Orientals as a group had an average nitrogen excretion of 167 mgm. per kgm. per day and the Caucasians, 146 mgm. Hence both from the standpoint of the total urinary nitrogen excretion and the excretion per kilogram of body weight the protein metabolism of these particular Orientals was not markedly different from that of their American college mates.

CONCLUSIONS

The basal metabolism of ten well-nourished, foreign-born Oriental women students (Chinese, Japanese, Korean and South Indian) who had

[1] Hetler (6) reports for eighty-five American college women an average 24-hour urinary nitrogen excretion of 7.69 grams.

been living for from one to three years in the United States, in an American college environment and partaking of an American college diet, averaged 12 per cent below the prediction standard and was lower than that of six American college mates. Comparison of the urinary nitrogen excretion of five of these Orientals and five of their American college mates (each pair living in the same college dormitory and eating at the same table) indicates that the Orientals were not subsisting upon an abnormally low protein diet and suggests that the low basal metabolism noted with these foreign-born Orientals under an American environment cannot be ascribed to a low protein metabolism.[2]

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[2] With two Europeans living in the tropics Radsma and Streef (7) found that an increase in protein intake resulted in an increase in basal metabolism. Hetler (6), Wang (8), and Tilt (9) on the contrary, found no apparent relation between the level of the protein intake and the basal metabolic rate of normal women.

THE RELATION OF THE PARAFLOCCULUS TO THE MOVEMENTS OF THE EYES

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The necessity of considering the flocculus and paraflocculus separately in any physiological experiment dealing with these parts of the cerebellum, has been shown by the comparative morphological studies recently summarized by Larsell (1934), by the embryological development of the cerebellum as described by Larsell and Dow (1935) and by the fiber connections of these two lobules which were recently determined by the author in work as yet unpublished. In studying the anatomy of the flocculus the often quoted work of Bárány (1914) on the function of the flocculus in rabbits was examined. These experiments were done on the paraflocculus and not on the flocculus. Stimulation of this part of the cerebellum was said by Bárány to cause various precise and intricate movements of the eyes. From these results he suggested that the "flocculus" (paraflocculus) in some way regulated the eye movements.

Many workers have observed eye movements following cerebellar stimulation from Ferrier (1886) on monkeys, rabbits, dogs and cats to Mussen (1927, p. 345) on cats. Horsley and Clark (1908) observed eye movements following stimulation of various parts of the cerebellar cortex and deep nuclei in monkeys, dogs and cats. They concluded that the movements observed by cortical stimulation were due to the spread of the current to the deep nuclei. Clark (1926) believed that all motor effects, including eye movements, produced by a stimulation of the cerebellum in monkeys and armadillos were caused by a spread of the current to extracerebellar motor centers. Lourié (1908) observed in dogs that the eye movements from a stimulation of the cerebellum near the mid-line were stronger than from a stimulation of its lateral parts.

In the present study, seven rabbits were used. They were anesthetized with dial (Ciba) given intraperitoneally in a dosage of 0.5 cc. of a 10 per cent solution per kilo. This was supplemented, when necessary, by 0.2 to 0.4 cc. of the same solution given intravenously. The animals were kept under as light anesthesia as possible during the experiment. For stimulation, a single dry cell battery (1.5 volt) was used. This was of

such strength that when the secondary coil of the inductorium was set at 9 cm. the faradic stimulus supplied by the bipolar platinum electrode could be easily felt on the tongue. In three animals the parts of the cerebellum to be studied were exposed by the use of a dental drill. In four animals the entire posterior surface of the cerebellum was exposed, artificial respiration being used. The paraflocculi, the lobuli paramedianus and the lateral parts of the pyramis were stimulated. It was found impossible to stimulate the flocculus in the rabbit because of the difficulties of surgical approach. In two of the animals given artificial respiration, the experiments were terminated by the death of the animal before all the above areas could be stimulated.

A spontaneous nystagmus was observed in some of the rabbits while they were going under the anesthetic. In a few cases in which the nystagmus persisted, stimulation of any part of the cerebellum stopped the nystagmus temporarily or replaced it by the type of movement of the eyes described below. Stimulation of the cerebral cortex or of the posterior surface of the medulla failed to inhibit this nystagmus in the one case in which these latter areas were stimulated.

The movements of the eyes are described as being in each of three planes, although they actually were the resultant of these individual components. The movements were slow to start, slow of execution, and the eyes slowly returned to their former position.

The strength of the stimulus necessary to produce a movement of the eyes decreased as the mid-line was approached and in each case, if the same strength of current was used, the eye movements were always greater when the stimulus was applied nearer the mid-line.

A stimulus with the secondary coil at 7 cm., or less, usually resulted in a spread of current to the facial or accessory nerves or their nuclei. Several times the eye movements resulting from stimulation before the dura was opened were compared to those afterward and no essential difference was noted. The eye movements were regularly greater on the homolateral side than on the contralateral side and in several instances they were confined to the same side.

There was considerable variation in the direction of the movements. Such variation was even observed in the same animal at different times during the experiment, especially if the strength of the stimulus was changed. At times stimulation, produced by thrusting the bipolar electrode into the deep cerebellar nuclei, caused a somewhat different movement than had resulted from superficial stimulation. This was never a complete reversal of the cortical movements as reported by Bárány (1914).

Rotation of the right eye almost always occurred in a clockwise direction while the left eye rotated in a counter-clockwise direction, with nearly the same frequency regardless of the side stimulated. This combination of movements was a forward rotation of each eye. Movements of the

outer pole of the eye in the horizontal plane were more frequently forward than backward. This tendency was more constant in the contralateral eye than in the homolateral eye. Movements in the vertical plane were slightly more frequently downward than upward in the homolateral eye. In the contralateral eye the movements were almost always upward.

The movements in one eye were usually in the opposite direction to movements occurring simultaneously in the other eye. Movements in rotation and in the vertical plane were more frequently in the opposite direction than were movements in the horizontal plane. This relationship between the movements of the two eyes showed about the same variability as was found in the direction of the individual eye movements.

No difference in the direction or the constancy of eye movements was detected when the movements observed in rabbits operated by one method were compared to those observed in rabbits operated by a different method.

CONCLUSIONS

1. Strong stimulation of the cerebellar cortex and relatively weak stimulation of the deep cerebellar nuclei results in eye movements in the rabbit. Of the cortical areas of the cerebellum tested the threshold of stimulation was lowest in the pyramis and highest in the paraflocculus.

2. These eye movements are somewhat inconsistent as to direction. A rotation of both eyes forward (right eye, clockwise; left eye, counterclockwise) is by far the most frequent rotatory movement observed in these experiments regardless of the side stimulated. The movements of the outer pole of the eye depend to some extent upon which side is stimulated.

3. The following observations would suggest that eye movements resulting from cerebellar stimulation in the rabbit may be caused by a spread of current to adjacent extra-cerebellar areas: *a*, the strength of the stimuli necessary for their production; *b*, the presence, in a few cases, of an equally good response with the dura intact; *c*, and the visible evidence of the spread of current to nearby motor nerves.

4. In contrast to Bárány, I find no specific relationship between the paraflocculus and the eye movements.

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AREA AND THE INTENSITY-TIME RELATION IN THE PERIPHERAL RETINA

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For short flashes of light and small areas of illumination the product of intensity (I) and exposure time (t) is constant for threshold excitation of the human eye (Bloch, 1885; Piéron, 1920; Braunstein, 1923). This relationship does not hold for exposure times beyond *ca* 0.05 second, and for a greater range of exposure times a more complicated expression is required. Blondel and Rey (1911) state that the equation $It = a + bt$ (where a and b are constants) gives a good description of the intensity-time effect over a range extending from 0.001 to 3.0 seconds. In examining the discharge of impulses in a single fiber of the optic nerve of *Limulus*, Hartline (1934) determined that below a certain critical duration, the energy ($I \times t$) necessary to produce a constant frequency of impulses is constant (C). Above this critical duration intensity alone seems to be effective and $I = Constant$. The presence of the critical duration had been demonstrated earlier in experiments by the same author (Hartline, 1928) on the grasshopper eye and by Adrian and Matthews (1927) on the eel eye.

In the experiments on *Limulus* the transition, at the critical duration, from the relation $It = C$ to $I = Const$ is fairly abrupt. On the other hand the data for the human eye show a continuous increase in threshold energy, so that the condition $It = C$ for short durations goes over gradually into $I = Const$ for long durations. It is significant that the experiments on *Limulus* dealt with a single sense cell with no central connections, while in the human eye we are of course dealing with effects due to the activity of a large population of sense cells. It is reasonable to expect that any sudden change in slope of the intensity-time relation which might be apparent for a single sense cell will be lost in the statistically determined effect from a great number of sense cells. For in the first place there is a distribution of properties among the individual sense cells (Hecht, 1927–28), and in the second place there exists a considerable degree of

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interaction among the nervous effects of sense cell activity (Adrian and Matthews, 1928; Granit and Harper, 1930; Beitel, 1934). Such processes as spatial and temporal summation which build up the total excitation to threshold must be considered.

These considerations led us to test the effect which a number of sense cells might have upon the form of the intensity-duration relation for threshold excitation of the human eye and to see whether by decreasing the area of the retinal image, with a consequent lessening of both statistical and interaction effects (Creed and Ruch, 1932), we might not approximate more closely to the type of relation found with single visual sense cells.

Apparatus and method. In one wall of the dark room provided for the subject was set an opal glass stimulus disk illuminated by an external source (concentrated tungsten filament). The intensity of illumination upon the stimulus disk was varied by means of Wratten Neutral Tint filters in conjunction with a Wratten Neutral Tint wedge (range 1 to 13) with balancing wedge. Constancy of the source was assured by reading the deflection of a galvanometer attached to a Weston "Photronic" cell which could be placed in the light beam, and adjusting the filament current accordingly.

Short flashes of light of known duration were obtained by means of the motor driven rotating disk shutter used by Hartline (1934) in his study of *Limulus*, and described by him. In this shutter, duration of flash is determined by speed of rotation of a "high speed" disk and angular aperture of the sector cut in it. Interchangeable disks provide a choice of sector apertures, and the speed of rotation can be controlled to within 3 per cent by a stroboscopic attachment. A low speed disk, in combination with a cam operated photographic shutter which opens before one flash begins and closes before the next one, enables the operator to obtain a single flash at any desired time. Since the light was brought to a small focus at the shutter before diverging to the stimulus disk, the full intensity of the flash is attained nearly instantaneously for most of the shutter settings; the exact time relations are given by Hartline.

The area of the stimulus disk was varied by means of circular diaphragms whose areas were 6.6, 0.86, 0.056, and 0.0012 sq. cm. Viewed through an opening in a large screen at a distance of 58 cm. these illuminated areas subtended visual angles of approximately 3°, 1°, 16′ and 2′ respectively. Fixation lights in the form of 1° red crosses (Tscherning no. 1 filters; brightness *ca* 0.3 millilambert) were placed on either side of the stimulus disk at a visual angle of 15° from its center. The stimulus patch was regarded monocularly; when the right eye was used the right fixation cross was illuminated; with the left eye, the left cross was lighted.

Before each series of determinations the subject was allowed to become dark adapted for 40 minutes. At a signal the subject regarded the fixa-

tion cross in foveal vision and was instructed to say whether or not he saw a flash of light when the shutter was opened. Thresholds were determined by the method of limits. Ordinarily, though not always, observations were made alternately with the left eye and right eye. Approximately 40 seconds were allowed to elapse between exposures of the same eye. After any one threshold was determined the subject was allowed to rest in the dark for three or four minutes while the necessary adjust-

TABLE 1

Log It values for four different areas

Intensity values are in terms of millilamberts

TIME	LOG *It*			
	2′ area (Subject G)	16′ area (Subjects M and G)	1° area (Subjects M and G)	3° area (Subjects M and G)
seconds				
0.00031		$\bar{5}.97$	$\bar{6}.75$	$\bar{6}.25$
0.00063		$\bar{5}.98$	$\bar{6}.77$	$\bar{6}.25$
0.001	$\bar{3}.29$			
0.00125		$\bar{5}.97$	$\bar{6}.78$	$\bar{6}.27$
0.002	$\bar{3}.28$			
0.0025		$\bar{5}.98$	$\bar{6}.77$	$\bar{6}.35$
0.004	$\bar{3}.28$			
0.005		$\bar{5}.99$	$\bar{6}.81$	$\bar{6}.34$
0.008	$\bar{3}.29$			
0.01		$\bar{5}.98$	$\bar{6}.83$	$\bar{6}.41$
0.016	$\bar{3}.30$			
0.02		$\bar{5}.99$	$\bar{6}.87$	$\bar{6}.45$
0.032	$\bar{3}.30$			
0.04		$\bar{4}.02$	$\bar{5}.05$	$\bar{6}.60$
0.064	$\bar{3}.28$			
0.08		$\bar{4}.11$	$\bar{5}.18$	$\bar{6}.70$
0.128	$\bar{3}.40$			
0.16		$\bar{4}.23$	$\bar{5}.26$	$\bar{6}.85$
0.256	$\bar{3}.59$			
0.32		$\bar{4}.40$	$\bar{5}.46$	$\bar{6}.99$
0.50	$\bar{3}.87$			
0.64		$\bar{4}.68$	$\bar{5}.63$	$\bar{5}.17$

ments in the apparatus were made. During each experimental period intensity thresholds were determined for twelve exposure time settings with a single area.

Results. Results of experiments made upon the two authors are presented in table 1. In figure 1 we have plotted these results, giving as ordinates the logarithms of the energy, $I \cdot t$, necessary for threshold excitation, and as abscissae the logarithms of the duration, t, of the flashes. The

experimental values for the three largest areas are averages of four determinations—two on each subject. (This averaging is legitimate, since

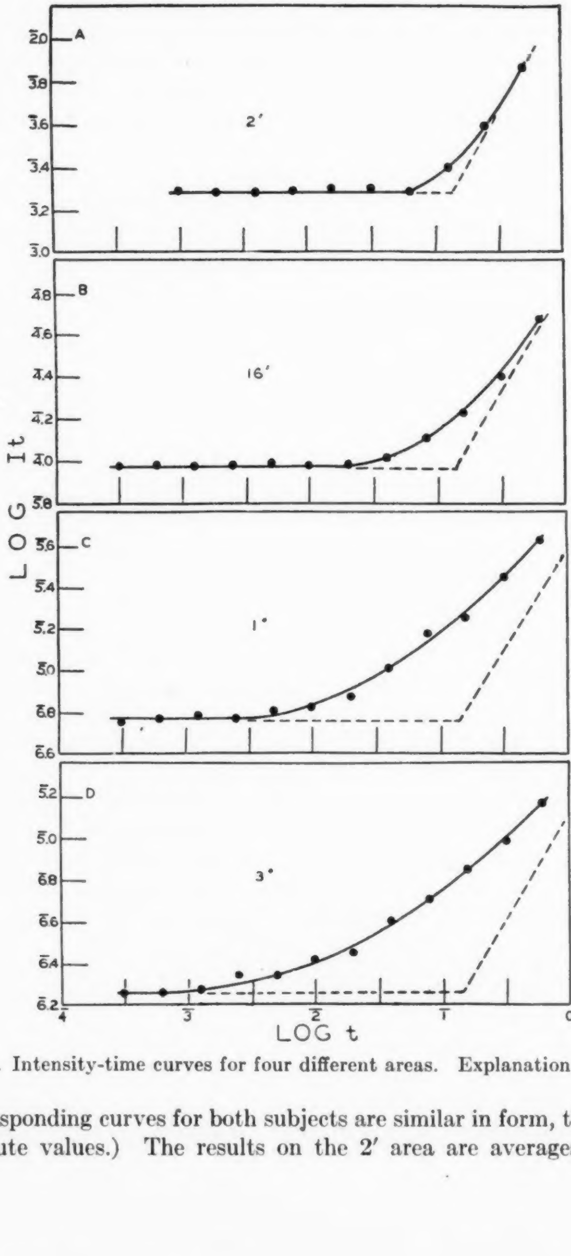

Fig. 1. Intensity-time curves for four different areas. Explanation in text

the corresponding curves for both subjects are similar in form, though not in absolute values.) The results on the 2′ area are averages of three

determinations on one of the authors (G) alone, and were obtained about eight months after the others.

It is seen in all the curves that for short durations the energy of flash $I \cdot t$ is constant; even in the lower curves the data approach a horizontal line ($I \cdot t = C$) as an asymptote. With long durations, however, the data for all the areas agree in showing an increased energy necessary for threshold, approaching asymptotically an inclined line of unit slope, which in this method of plotting represents the relation $I = Const.$ We have drawn in these asymptotes (dashed lines) for the four curves of figure 1. The inclined line of unit slope is drawn to fit the data of the upper curve; for the other curves it is so drawn as to intersect the horizontal asymptote at the same duration as in the upper curve.

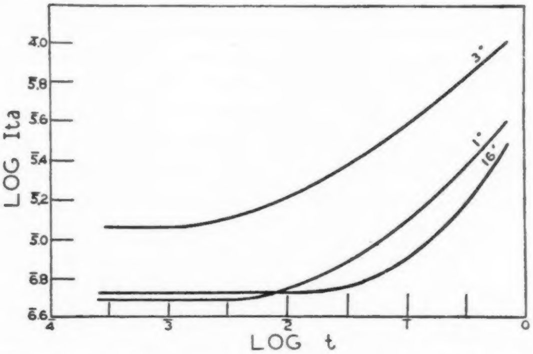

Fig. 2. Intensity-time-area curves for the three largest areas. Explanation in text.

The effect of area of retinal image upon the shape of the intensity-duration curve is quite striking. With the largest area there is a very gradual increase in threshold energy with duration of flash, the data being fitted by a gently rounded curve between the two asymptotes. As area is decreased, it is seen that this curve becomes more angular, and that the energy necessary to excite at the shortest duration is still sufficient at higher and higher durations. With the smallest area the threshold energy is constant over a considerable range of durations, and the upward bend is quite sharp, so that the relation $I = Const$ holds for shorter durations than with the larger areas.

It is to be noted that the absolute value of the threshold energy per unit area ($I \cdot t$) is greater the smaller the area of the retinal image. This is another example of the familiar reciprocity influence of area and intensity. In figure 2 we have plotted three of the curves in terms of total energy ($I \cdot t \cdot a$, where a is the area of the stimulus disk). It is seen that for the two smallest areas, 1° and 16′, the curves agree quite closely at

short durations, so that we may say that for small areas and short durations of flash the energy, $I \cdot t \cdot a$, necessary for threshold excitation, must be constant. (Moreover, G's values for the smallest area, 2′, agree quite satisfactorily with his values for 16′ and 1°.) With the largest area, however, definitely more total energy is required at all durations to produce threshold excitation.

Discussion. The validity of the reciprocity relationship between intensity and duration for threshold stimulation of the human eye is shown by our results for flashes of short duration. This is in complete agreement with the findings of previous workers, as is also our observation that with longer durations more energy is required, and that eventually the threshold depends only upon intensity. Our principal finding is the observation that area of retinal image markedly affects the shape of the curve relating threshold energy to duration of flash, and that with sufficiently small areas the transition from the relation $I \cdot t = C$ to $I = Const$ is much more abrupt than with large areas. Our results for the human eye thus come directly into line with those of Hartline for the single sense cell of the *Limulus* eye, where the transition is quite abrupt, and is characterized by a clearly marked critical duration closely akin to McDougall's (1904–5) "action time." Although there will remain a statistical element in the determination of threshold even with the smallest areas, due to eye movements, etc., it is significant that when this element, together with interaction effects in the nervous layer of the retina, is minimized by reducing the number of end organs illuminated, the intensity-time relation approximates more and more closely to that found for the single sense cell.

The confirmation which our results provide of the familiar reciprocal relation between area and intensity is in harmony with other observations (Adrian and Matthews, 1927, 1928; Granit and Harper, 1930; Beitel, 1934). We may interpret it as due to the summation of excitatory processes in the synaptic layers of the retina, inferring that the threshold effect must take place in the nervous paths beyond the layer of rods and cones.

While it is not understood why this summation should result in a close reciprocal relation between intensity and area, we may accept it as experimental fact, and interpret the failure of this relation with large areas as due to incomplete spatial summation. Thus when a great number of central nervous neurones is excited the greatest degree of excitation will be in the neurones in the center of the group, since these neurones receive the greatest benefit from the excitation over the interconnecting pathways. The threshold level of excitation will consequently be determined by the activity of this group. With large areas more fibers are brought in which have few convergence points in common with the central group and these peripheral fibers contribute little to the summed central excitation; hence a disproportionate amount of energy will be required for the threshold effect.

The question as to why there is an increase in the relative energy requirement at shorter and shorter exposure times as area increases is an important one, but one which if treated would necessarily have to be discussed on a highly speculative level. In addition to considering the possibility of a distribution of critical durations in the separate fibers, we should probably have to consider the increased temporal dispersion of excitation which occurs with an increase in area.

SUMMARY

Intensity-time curves have been obtained for four different circular areas (diameters 3°, 1°, 16′, and 2′) of retinal image in the periphery of the human eye. The range of durations extends from 0.00031 to 0.64 second.

For short durations, the energy per unit area ($I \times t$) must be constant to produce threshold excitation. For longer durations, more energy is required, and the condition for threshold excitation finally becomes $I = Const.$ The absolute intensity threshold for any duration increases as area decreases. These findings confirm the results of previous workers.

A decrease in the area of retinal image results in a more abrupt transition from the condition $I \times t = C$ to $I = Const$, and with the smallest area the data approach the form which has been described for the activity of the single visual sense cell from the eye of *Limulus*.

These findings are interpreted as indicating that the excitation of the photoreceptor of the human eye is characterized by a critical duration similar to that found in the *Limulus* sense cell. The effect of area in masking this critical duration is interpreted as due to statistical distribution of properties among a large number of sense cells, and to the increased interaction effects associated with large areas. There is a discussion of certain aspects of these interaction effects.

We wish to express our appreciation to Doctors D. W. Bronk and H. K. Hartline for the facilities placed at our disposal and for valuable criticisms and suggestions throughout the course of the work.

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THE INFLUENCE OF EPINEPHRINE ON THE BLOOD SUGAR, LACTIC ACID AND INORGANIC PHOSPHORUS OF COMPLETELY HYPOPHYSECTOMIZED DOGS[1]

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The intracranial approach for the removal of the hypophysis of the dog involves 3 distinct manipulations: 1, craniotomy and incision of the dura; 2, retraction of the temporal lobe, a procedure that brings the hypophysis into view, and 3, interference in the region of the base of the brain, a procedure that consists in the excision of the gland. In a previous communication the insulin sensitivity of 3 groups of dogs in which the various stages had been performed was compared with that of the normal (1). An increased sensitivity to insulin was found not only in dogs in which all 3 manipulations (i.e., complete hypophysectomy) had been performed, but also in those animals in which the gland—although left intact and subsequently shown to be normal by serial sections of the whole hypophyseal region—had been exposed to view by cerebral retraction as far as the sella turcica. Since insulin hypoglycemia accelerates the output of epinephrine from the adrenal gland, the action of insulin on the blood sugar is complicated by that of epinephrine, a hormone that in itself influences carbohydrate metabolism even in doses that have no effect on blood pressure. In view of the striking capacity of epinephrine to counteract hypoglycemia, it seemed a reasonable inference that the increased insulin sensitivity observed in dogs that had suffered hypophyseal interferences might be due to a diminished release of epinephrine during hypoglycemia or else to the failure of epinephrine—even though released in sufficient amounts—to bring about its normal carbohydrate reactions. In the present investigation, therefore, the response to epinephrine of the dogs that gave an increased insulin reaction was compared with that of normal animals. As an index of the dog's response to epinephrine, 3 blood constituents were studied: blood sugar, inorganic phosphorus and lactic acid, the responses of all 3 of which involve both liver and muscle. Previous work has dealt with one of these constituents, namely, blood

[1] The expense of this investigation was defrayed in part by a grant to one of us (I. L. C.) from the Research Board of the University of California, Berkeley.

sugar, and the results obtained have been contradictory. Thus while Aschner (2), Braier (3), and Corkill, Marks and White (4) found a decreased epinephrine glycosuria or hyperglycemia in hypophysectomized animals, other investigators (5, 6, 7) have reported increased responses to epinephrine in animals in which the pituitary glands had been completely or partially excised.

Experimental. The dogs used in the present investigation, both those operated upon and normal, have already been reported in connection with a study of their insulin sensitivity (1). In order that the response to both insulin and epinephrine may be compared, the nomenclature previously employed has been kept. Three groups of dogs were studied.

1. *Hypophysectomized dogs.* The various manipulations involved in hypophysectomy by the intracranial route have been previously described (1, 8). Detailed necropsy findings of the 7 dogs studied have been noted elsewhere (1). There was a complete absence of anterior, posterior and intermediate lobes of the gland, as well as of all stalk tissue.

2. *Control dogs.* Since, in addition to excision of the gland itself, hypophysectomy by the intracranial route involves 2 manipulations designed to bring the hypophyseal region into full view, namely, craniotomy and retraction of the right temporal lobe, 4 dogs were subjected only to the 2 latter procedures. The details of this operation have been previously described (1). Serial sections of the whole region of the pituitary in the 4 dogs studied showed this gland to be intact and normal.

3. *Normal dogs.* The 4 dogs of this group suffered no operative interference with either cranium or brain.

The dietary treatment of the 4 groups of dogs has been reported elsewhere (1). Following the removal of a postabsorptive sample of blood, epinephrine was injected subcutaneously in doses of 0.10 mgm. of 1:1000 solution per kilo of body weight. It has been shown that the administration of the hormone in such doses by the subcutaneous route does not affect the blood pressure in dogs (9).

The methods employed for the determination of blood sugar and inorganic phosphorus have been previously described (1). For the estimation of lactic acid Wendel's modification (10) of the method of Friedmann, Cotonio, and Shaffer was used. All determinations were made in duplicate, and the results recorded are the means of closely agreeing values.

Results. The 4 normal dogs were maintained on the experimental diet for 4 to 4.5 months, and during this period their reactions to epinephrine were tested on 9 different occasions. The 7 hypophysectomized dogs were tested 13 times for their response to epinephrine. A period varying from 4 to 12 weeks was permitted to elapse after the operation before the first injection of epinephrine was made, while the latest study was done approximately 4.5 months after excision of the gland. The 4

animals of the control group received epinephrine on 7 occasions at intervals varying from 6 to 16 weeks following the operation.

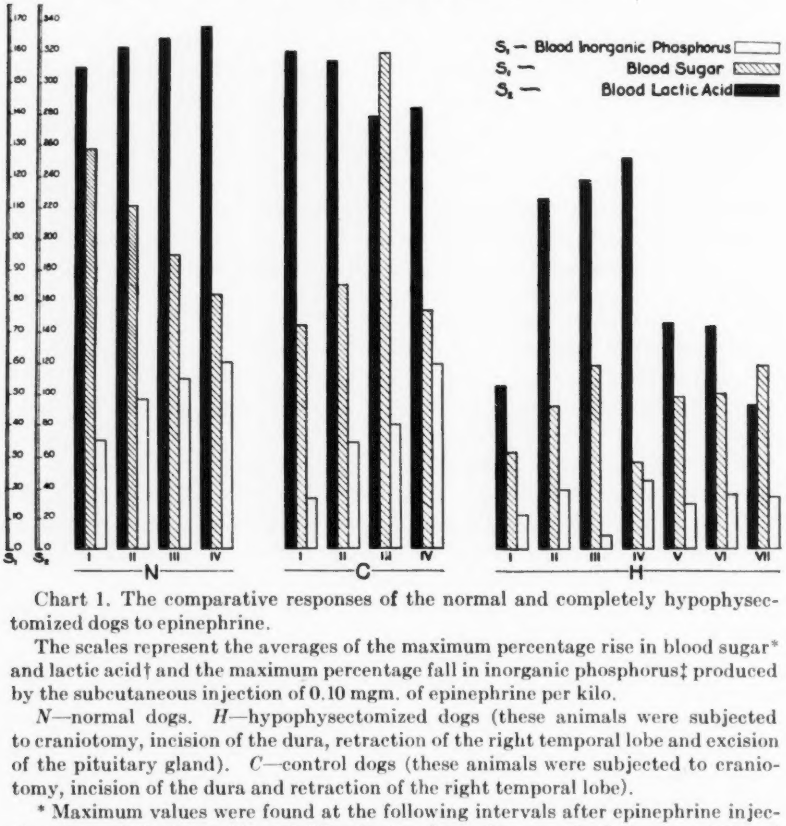

Chart 1. The comparative responses of the normal and completely hypophysectomized dogs to epinephrine.

The scales represent the averages of the maximum percentage rise in blood sugar* and lactic acid† and the maximum percentage fall in inorganic phosphorus‡ produced by the subcutaneous injection of 0.10 mgm. of epinephrine per kilo.

N—normal dogs. *H*—hypophysectomized dogs (these animals were subjected to craniotomy, incision of the dura, retraction of the right temporal lobe and excision of the pituitary gland). *C*—control dogs (these animals were subjected to craniotomy, incision of the dura and retraction of the right temporal lobe).

* Maximum values were found at the following intervals after epinephrine injection: normal, between 105 and 380 minutes; hypophysectomized, between 180 and 330 minutes; control, between 160 and 380 minutes.

† Maximum values were found at the following intervals after epinephrine injection: normal, between 120 and 240 minutes; hypophysectomized, between 115 and 235 minutes; control, between 110 and 230 minutes.

‡ Minimum values were found at the following intervals after epinephrine injection: normal, between 60 and 175 minutes; hypophysectomized, between 60 and 180 minutes; control, between 115 and 170 minutes.

The blood sugar, inorganic phosphorus and lactic acid were followed at repeated intervals for a period of 8 hours after the subcutaneous injection of epinephrine. Since the lack of space would not permit the recording of individual results, the averages of maximum percentage changes

produced by the hormone in the 3 blood constituents in each of the 3 groups of dogs are compared in chart 1. In the 3 groups of dogs studied, the most rapid response to epinephrine, as well as the greatest relative change, occurred in lactic acid. Although the degree of the reaction to the hormone varied among the 3 groups, the duration of the effect was roughly the same. Thus at the 6-hour interval after the epinephrine injection, inorganic phosphorus of the blood had returned to normal or slightly above normal, whereas, in the case of blood sugar and lactic acid, values above normal were usually found at this time interval.

Discussion. As judged by the changes produced in 3 blood constituents, namely, the rise in glucose and lactic acid and the fall in inorganic phosphorus, completely hypophysectomized dogs (H group) are capable of responding to epinephrine. Although definite, this response is nevertheless somewhat diminished when compared with that of normal dogs. Since responses comparable with that of the normal were obtained in dogs (C group) in which craniotomy and cerebral retraction had been performed—a procedure that involves all manipulations of hypophysectomy up to, but not including, excision of the gland itself—, it may be concluded that the diminished epinephrine reaction is the result of the removal of the gland only. The failure of epinephrine to affect the blood sugar, lactic acid and inorganic phosphorus in the hypophysectomized dog to the same degree as in the normal cannot be ascribed to differences in caloric ingestion, for both operated and normal animals were maintained under the same dietary conditions throughout the whole period of observation, the hypophysectomized as well as the normal dogs possessing good appetites and in most cases ingesting all food within a short time after it was served. The abnormal epinephrine reaction of the hypophysectomized dog is apparently a permanent characteristic, for a decreased response was found as late as 4 months after removal of the gland.

Carbohydrate cycle. Although glucose of the blood is derived directly from liver glycogen, it is now well established that the quantities of glycogen normally present in the liver will not account for the long sustained hyperglycemia observed during epinephrine action (11, 12). In addition to mobilization of liver glycogen, two other mechanisms induced by this hormone are essential in the production of epinephrine hyperglycemia: 1, an impaired utilization of blood sugar in the peripheral tissues, and 2, a new formation of liver glycogen at the expense of muscle glycogen. Since it is by way of blood lactic acid that by far the major part of the glycogen lost by muscles during epinephrine action is transported to the liver (13), it is clear that the most important factor in the removal of lactic acid from the blood is its conversion to liver glycogen. It was found in the present investigation that, after maximum values had been attained, the blood lactic acid dropped fairly rapidly in the hypophysectomized

dogs. This suggests that, under the influence of epinephrine, the formation of liver glycogen from blood lactic acid proceeds in the complete absence of the pituitary gland. Since this conversion makes muscle glycogen a source of blood sugar, the rate of muscle glycogenolysis determines, in part, the degree of hyperglycemia during prolonged epinephrine action. The decreased response of the blood sugar to subcutaneously injected epinephrine observed in the hypophysectomized dogs is therefore due in part to the decreased rate of lactic acid formation, which was also observed in these dogs under the influence of this hormone. This probably is not the only factor involved, for despite the presence of glycogen in the liver Corkill, Marks and White (4) found that the blood sugar of some of their hypophysectomized rabbits was not affected by epinephrine. They therefore concluded that, in the absence of the hypophysis, glycogen of the liver becomes resistant to mobilization by epinephrine. In passing it should be noted that in contrast to the findings of these workers in the *rabbit* (4), a complete failure of the blood sugar of the hypophysectomized *dog* to respond to the hormone was not observed in the present study.

Inorganic phosphorus. Epinephrine leads to a temporary increase in hexosephosphate in muscle (14), a process that accounts for the decrease in blood inorganic phosphorus observed under the influence of this hormone. Since muscle hexosephosphate is the precursor of blood lactic acid, it might be expected that the rate of glycogen breakdown in muscle would determine the rate of hexosephosphate formation. Indeed, it has been shown that the blood lactic acid curve runs parallel with the rise and fall of hexosephosphate content of muscle during epinephrine action (14). The abnormally low response of the blood inorganic phosphorus to epinephrine observed in the hypophysectomized dogs may therefore be regarded as evidence that less hexosephosphate is being formed in the muscles of these animals than in normal dogs—a conclusion that is in keeping with the finding that the rise in the blood lactic acid was also less pronounced in dogs after removal of the pituitary glands.

SUMMARY

1. The effects of epinephrine on blood sugar, lactic acid and inorganic phosphorus, the responses of all 3 of which involve both liver and muscle, were compared in 3 groups of dogs: 1, normal; 2, completely hypophysectomized, and 3, dogs designed to serve as controls for the second group.

2. As judged by the 3 blood constituents, completely hypophysectomized dogs are capable of responding to epinephrine. The responses, however, were diminished as compared with those of normal and control animals.

3. The significance of the abnormal epinephrine reaction of the completely hypophysectomized dog is discussed.

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THE CALCULATION OF CARDIAC OUTPUT AND EFFECTIVE PERIPHERAL RESISTANCE FROM BLOOD PRESSURE MEASUREMENTS WITH AN APPENDIX ON THE SIZE OF THE AORTA IN MAN

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This subject has been of general interest since 1896, and recently has given rise to considerable discussion in the literature, particularly in regard to the validity of the equations introduced by Broemser and Ranke (1930); Frank (1930); Lauber and Pryzwara (1930).

Hürthle, in 1896, described a method for measuring blood pressure in man and stated then that "the fall in pressure in the arteries in diastole is dependent on the rate of flow from the arteries into the veins only, therefore the velocity of the fall of pressure is a direct measure of the resistance opposed to this flow." Hürthle, therefore, recognized at this date that an adequate analysis of blood pressure changes would provide a measure not only of the blood flow, but of the effective resistance offered to this flow. Frank (1899) discussed mathematically the relationship of flow to pressure and first emphasized quite clearly the necessity of distinguishing the outflow from arteries to veins in diastole from the more complicated condition of systole, where the pressure changes in the arteries are determined by the balance between inflow and outflow. Frank pointed out that the distensibility of the system was an important factor, and that this value must vary during the pulse cycle, and that similarly the effective resistance in the periphery would be reduced in the systolic phase owing to the stretching of the vessels by the increase in pressure. Hürthle (1901) demonstrated that systolic outflow was increased above that in diastole more than in arithemetrical proportion to the rise in pressure, and this was established by Hürthle (1919), Fleisch (1919), and by Wagner (1928). Bazett (1927) showed that these data indicated that flow was probably proportional to the 3/2 power of the pressure, but a more direct attack on this problem by Whittaker and Winton (1933) indicated that the relationship was apparently represented in the dog by an equation of the type:

$$V = K(P - a)$$

[1] Travelling Fellow of the Rockefeller Foundation.

where V is the volume flowing in unit time, P is the pressure, K and a are constants, and where a has the approximate value of 20 (if pressure is expressed in millimeters of mercury). The absence of a simple proportion between flow and pressure, which is abundantly proven in the literature, has been neglected in Broemser and Ranke's equations.

Broemser and Ranke have based their equations on the distensibility of the vessels as estimated from the pulse wave velocity between the subclavian and femoral and the duration of systole. On these values the length of the arterial tree distended during systole is calculated, and, from the volume so estimated as stored in the arterial tree during systole, the total stroke volume is deduced. This theoretical foundation neglects the experimental fact that the pulse wave velocity is much more rapid in the smaller peripheral than in the larger central vessels (Bazett and Dreyer, 1922) so that, in point of fact, in over 100 records on subjects of different age, size, and experimental condition we have never observed a single case in which the pulse wave had not commenced in the dorsalis pedis artery at the time the aortic valves closed. Often the pulse wave in the dorsalis has at this time almost reached its peak (see figs. 1 and 2). Since, therefore, the whole arterial tree is partially or completely distended in systole, the theoretical basis of their equation is unsound. Their equation has also been criticized by Frank (1930) and by Frank and Wezler (1931) on the additional grounds that indirect measurements of blood pressure fail to give true lateral pressures, and that the physical factors in the circulatory system are too complex to be analysed on physical principles.

Consequently, the equations suggested by Broemser and Ranke have only been given casual attention, though such attention that has been given them suggests that they are inadequate; an equation of a different type dependent on the calculation of the total stroke volume from the diastolic outflow, as originally suggested by Frank (1899) and von Recklinghausen (1906), has been adopted. No attempt has been made to achieve any exact physical analysis, but approximate empirical equations, based on some of the fundamental factors involved, have been tested against measurements of stroke volume by some respiratory method, and the equations have then been gradually modified, as such checks have demonstrated failure of the earlier equations. Preliminary accounts were published by Bazett (1932) and by the present authors (1934). Considerable attention also has been directed to establishing the accuracy of the method used for blood pressure measurement (Bazett, Laplace and Scott, 1935 and earlier papers). Since Hürthle (1923) demonstrated that the value of Young's modulus in the living dog was (in kilograms per square millimeter) 0.018 for the arch of the aorta; 0.118 for the carotid; and 0.178 for the femoral, the aorta must be considered as not only the largest

vessel, but also that with the greatest distensibility; consequently, emphasis has also been placed on the measurement of the pulse wave velocity in the arch of the aorta by the comparison of the apex beat with the subclavian pulse, as well as on measurements of such velocities in more peripheral vessels. It has been recognized that the deduction of the time the aortic valves open from apex beat records is often precarious, and various means have been adopted to circumvent these difficulties, and a number of other methods of detecting the opening of the aortic valves have also been put to an experimental test, so that probably a more reliable method can be substituted for apex beat records. This improvement will be reported later; it in no way modifies the principles or method of calculation. In the series of experiments here reported the apex beat was utilized, and some of the discrepancies observed may have arisen from errors of this source.

Most comparisons have been made with cardiac output estimates by Grollman's method; the two sets of measurements were made as nearly simultaneously as possible.

METHODS. The subject lay on a couch on his left side, and was kept comfortably warm; he remained quiet for 30 to 45 minutes before records were taken. All basal records were taken in the morning. Pulse wave velocity and blood pressure estimations were made, oxygen consumption was determined by a Benedict-Roth apparatus, and finally the arteriovenous oxygen difference was determined by acetylene.

For *pulse wave velocity* determinations simple or glycerine tambours of 4 cm. diameter were held over the apex and subclavian by elastic bands, over the femoral by a U-frame of flexible brass, and over the brachial and dorsalis pedis arteries by straps. All these tambours connected with tubing 1.5 meter in length to recording Frank capsules provided with concave mirrors. The camera carried paper of 12 cm. breadth with a speed of 4 to 10 cm. per second. Measurements of the distances were made as follows: a, the shortest distance between the angle of Ludwig on the sternum (junction of 2nd rib) and the center of the subclavian tambour; b, from the angle of Ludwig to the tip of the acromion and so to the center of the brachial tambour with the arm extended; c, from the same point to 2.5 cm. below the umbilicus and so to the center of the femoral tambour; d, from the femoral tambour to the dorsalis pedis tambour. On the basis of measurements on a single autopsy, and the previous estimates made by Bazett and Dreyer (1922) and by Matske, Priestley and Sands (1923), and on the assumption that the pulse wave travelled normal to the cross section of any tube, and, consequently, tended to follow the outer wall of curves such as that of the aorta, the following values were assigned to the actual distances travelled by the pulse waves; from heart to subclavian 1.6 a; from subclavian to brachial b−a; from

subclavian to femoral 1.25 c − 1.6 a; from femoral to dorsalis pedis d. Measurements of the records were made from the beginning of the upstroke of the pulse curve. In any experiment estimates were made of the pulse wave velocities from heart to subclavian, subclavian to femoral, subclavian to brachial, and, in all the later experiments, of femoral to dorsalis pedis. The pulse wave velocity values recorded represent the mean values of at least 10, often of 20 or more, cycles, chosen to include the whole respiratory cycle.

To assist in the interpretation of the apex beat records, the apex tambour was moved in different records to different positions, and usually some one of these was easy to interpret and provided a key to the others. Figures 1 and 2 indicate the sort of records obtained, and the variation in the type of apex beat record that may be seen in a single experiment and even in successive cycles (respiratory variation) in a single record. In figure 1, the point marked 2 is fairly definitely marked, either by an increased frequency of secondary waves or by a sharp upstroke, and this point probably indicates the start of expulsion; the point marked 1 similarly probably indicates the start of ventricular contraction. Similarly, in figure 2 the points 1 and 2 have been taken as representing the start of ventricular contraction and expulsion respectively. It should be mentioned that the factors concerned in the apex beat are so complex that with certain positions of the tambour the beginning of expulsion may be indicated by a sharp downstroke rather than an upstroke.

Blood pressures have been taken by the method of Bazett and Laplace (1933), and the calculations are based on the lateral systolic, dicrotic and diastolic pressures so determined. At least 3 records of compression and decompression were made for all basal estimates, so that the value used represents the mean of at least 6 estimates. If respiratory variations were marked, a larger number of records was used. Standardization of the recording manometer was effected automatically by employing a mercury manometer in parallel with it; this mercury manometer was fitted with platinum contacts so that the levels of 50 and 150 mm. Hg were registered on each curve by a signal magnet. For blood pressure records the paper movement was 1 to 2 cm. per second. The pressures as read were checked by comparison with the brachial pulse curves. The heights of the main curves and of the dicrotic wave in the series of waves of a respiratory cycle were measured, and from these measurements the pressure of the dicrotic wave was calculated on the assumption that the peaks and troughs of the waves could be assigned the values found for lateral systolic and diastolic pressure. The mean of the experimentally measured and calculated estimates was taken to represent the dicrotic pressure.

The *mean pressure* was estimated by cutting out the brachial pulses of one respiratory cycle, and weighing them. In this way their area and mean height were determined; if the average maximum height of the curves be represented by *a*, the mean height of the curves by *b*, diastolic

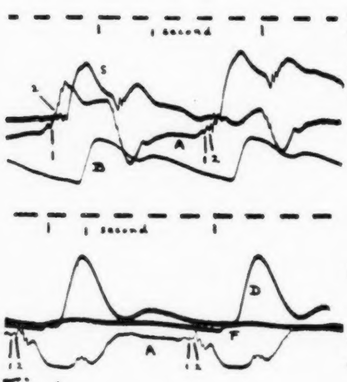

Fig 1.

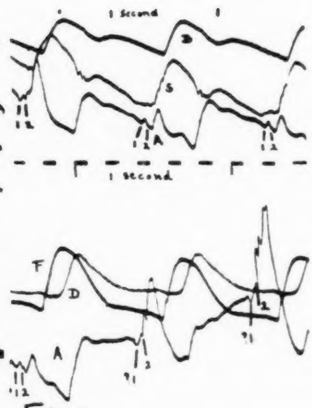

Fig 2.

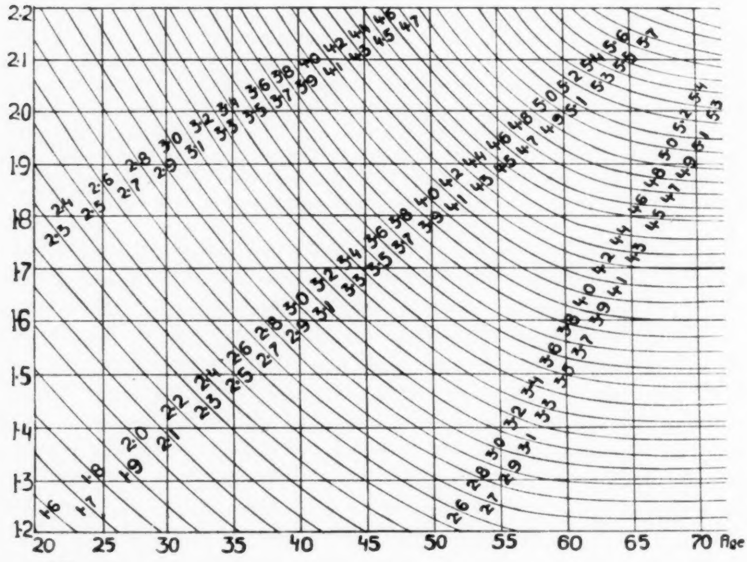

Fig. 3

Fig. 1. Subject 1, basal, upper record; apex beat, *A*; subclavian, *S*, and brachial, *B*. Lower record; apex beat, *A*; femoral, *F*, and dorsalis pedis, *D*. Time in $\frac{1}{5}$ second. The times were from heart to subclavian 0.063, subclavian to brachial 0.076, apex to femoral 0.152 and femoral to dorsalis 0.083 second; isometric contraction period 0.032 second. Estimated pulse wave velocities on this record were 1, 3.30; 2, 5.18; 3, 5.40, and 4, 11.02 meters a second.

Fig. 2. Subject 5, lying after meal. Records labelled as in figure 1. The times were: heart to subclavian 0.056, subclavian to brachial 0.056, apex to femoral 0.123, and femoral to dorsalis pedis 0.103, and isometric contraction period 0.037. Estimated pulse wave velocities were 1, 3.15; 2, 7.95; 3, 7.00, and 4, 8.15 meters a second.

Fig. 3. Estimated relationship of internal cross-sectional area of the ascending aorta to surface area and age; abscissae—age in years; ordinates—surface area in square meters; contour lines—area of aorta in square centimeters.

pressure by D, systolic pressure by S, and mean pressure by M, then $M = D + b/a\ (S - D)$.

The *relative durations of systole and diastole* were also arbitrarily determined from the *brachial pulse*; the precaution of making estimates from a whole respiratory cycle was followed as usual. The "diastolic" (d) period was measured from the top of the dicrotic wave to the end of the cycle; the rest was considered as "systolic" (s). The division is artificial, but the diastolic period so defined is one relatively free from the complexities of secondary waves, and there is an advantage in that the pressure measurements are also made on the brachial pulse.

From the mean pressure and the ratio of the s period to the cycle the *mean pressures in these s and d periods* were estimated. The pulse curve usually shows a relatively steady fall of pressure in diastole, and the mean pressure of the d period may then be taken as half-way between the dicrotic and diastolic pressures. In the rare cases where the curve was far from linear, the mean value was determined by weighing. The mean pressure of the s period can then be calculated. If M_d is the mean pressure in the d period, M_s that in the s period, s/c and d/c the ratios of these s and d periods to the cycle, and M mean pressure as before, then

$$M = s/c\ M_s + d/c\ M_d$$

Calculations of volume changes were made on the basis of Bramwell and Hill's (1922) modification of Moen's equation—namely, that the percentage change in volume per millimeter of mercury increase in pressure $= \frac{12.7}{v_2}$, where v is the pulse wave velocity in meters per second. Consequently, it was essential to estimate the volumes of the vessels which vary with the size of the individual and also with age. The probable internal cross-sectional area of the ascending aorta for subjects of different size and age has been estimated from data in the literature and the values so estimated are indicated in figure 3. The method of derivation of these curves is of some interest in regard to age changes and will, consequently, be described in an appendix to this paper. Volumes were assigned to the four arterial sections on the basis of the curves of figure 3, the standing height, and the surface area. Volume 1 ($\bar{V}_1$ of the equations) represents the assumed volume at the end of diastole of the aorta and its branches up to a distance equal to that of the third part of the subclavian; volume 2 ($\bar{V}_2$) represents the volume of the descending aorta, and the iliacs, and its other large branches with the exclusion of that section nearer to the heart than the subclavian; volume 3 ($\bar{V}_3$) represents the volume of blood contained in the vessels between the subclavians and the brachials at the elbow and in other vessels of the upper part of the body with similar pulse wave velocities; volume 4 ($\bar{V}_4$) represents the volume contained in

the vessels of the two lower limbs and also any blood in the upper extremities, splanchnics, or elsewhere, contained in vessels with pulse wave velocities similar to that observed in this section. The volumes of $\bar{V}_1$ and $\bar{V}_2$ have been derived on the basis of autopsy data, and have been considered to vary both with size and age; (they have been slightly modified on empirical grounds to give a better fit with the acetylene data, for these volumes should not be the real volumes of the aorta but the effective volumes, i.e., the volumes which would act like those of the aorta if contained in tubes in which the pulse wave velocities were constant throughout the lengths of the tubes). The volumes $\bar{V}_3$ and $\bar{V}_4$ have been assumed to vary only with size and not with age (see appendix); the values assigned are entirely empirical and are based only on the capacity of the equations to give stroke volumes which agree with those calculated by the other method, and on the assumption that the volume $\bar{V}_4$ is likely to be considerably greater than $\bar{V}_3$.

The volumes used in the calculations are as follows:

$$\bar{V}_1 = Q \times 0.12\ \mathrm{H} \qquad \bar{V}_2 = Q \times 0.15\ \mathrm{H}$$
$$\bar{V}_3 = A \times 0.065\ \mathrm{H} \qquad \bar{V}_4 = A \times 0.34\ \mathrm{H}$$

where Q is the cross-sectional area of the aorta determined from figure 3, H the standing height in centimeters, and A is the surface area in square meters by the Du Bois height-weight formula.

Then applying the principles developed by von Rechlinghausen (1906), the volume of blood leaving the arterial tree and the distensibility of the system are the sole determinants of the fall of pressure in the d period, so that:

$$V_d = \frac{12.7\,(Z - D)}{100}\left(\frac{\bar{V}_1}{v_1^2} + \frac{\bar{V}_2}{v_2^2} + \frac{\bar{V}_3}{v_3^2} + \frac{\bar{V}_4}{v_4^2}\right)$$

where V_d is the blood leaving in diastole, Z is the dicrotic, D the diastolic pressure, $\bar{V}_1$ to $\bar{V}_4$ the volumes as described and v_1 to v_4 the velocities of the pulse wave for the corresponding sections. In the earlier experiments measurements of the pulse wave velocity from femoral to dorsalis pedis were not made; in these (equation 2) $\dfrac{\bar{V}_4}{{v_3}^2}$ was used to replace the last two values of the above equation.

But the blood leaving the arterial tree in the s period should be related to that leaving in the d period according to the relative durations of, and the relative pressures in, these two periods, and it is immaterial that the s period does not represent the true systole. If the data of Whittaker and Winton (1933) be accepted then

$$V_s = V_d \cdot s/d\left(\frac{M_{s-20}}{M_{d-20}}\right)$$

where V_s is the volume leaving in the s period. Then the total stroke volume (V) = $V_d + V_s$ and the cardiac output per minute $V_t = V \cdot F$ where F is the pulse frequency per minute. Since the mean pressure is determined by the cardiac output and by the mean resistance offered by the arterioles and other peripheral vessels to blood flow, the effective value of this resistance (R) may be calculated from the relation of the mean pressure to the cardiac index:

$$R = k \frac{M}{V_t/A}$$

where A is the surface area in square meters, and k is a constant. If k be given the value of 3, the value of R is, in most young subjects when warm and under basal conditions, approximately 100 (80 to 120).

One example of the calculations may be given, when both methods agreed in giving subnormal values.

Subject 6: surface area 1.69. Height 167.2 cm. Age 30. October 26, 1934. Effective temp. (low) 17.3°C.

Average pressures: S 115.9 mm. Hg. Z 96.4 mm. Hg. D 74.6 mm. Hg. F 65.4 per min.

Average pulse wave velocities: (1) 3.12 (2) 7.79 (3) 6.31 (4) 8.53

s/c 0.455.

The average maximum height of the pulse curves was 25.4 mm.; of the dicrotic wave 13.2 mm.; $Z - D$ was by oscillometer 21.8, by pulse curves $\frac{13.2}{25.4} \cdot 41.3 = 21.5$ and its mean value 21.7.

The mean height of the pulse curves was 10.15 mm.

$$M = 74.6 + \frac{10.15}{25.4} \times 41.3 = 91.1 \text{ mm.}$$

$$M_d = 74.6 + \frac{21.7}{2} = 85.4 \text{ mm.}$$

$$0.455\, M_s = 91.1 - 0.545 \times 85.4 \quad \text{and} \quad M_s = 97.9 \text{ mm.}$$

The assumed cross-sectional area of the aorta was 2.58 sq. cm. and the arterial volumes 1–4 consequently were: 51.8, 64.7, 18.4, and 96.0 cc.

Then

$$V_d = 21.7 \times \frac{12.7}{100}\left(\frac{51.8}{(3.12)^2} + \frac{64.7}{(7.79)^2} + \frac{18.4}{(6.31)^2} + \frac{96.0}{(8.53)^2}\right) = 22.6 \text{ cc.}$$

$$V_s = 22.6 \times \frac{0.455}{0.545} \times \frac{97.9 - 20}{85.4 - 20} = 22.5 \text{ cc.}$$

$$V = 22.6 + 22.5 = 45.1 \text{ cc}; \qquad V_t = 45.1 \times 65.4 = 2.95 \text{ liters per mm.}$$

$$\text{The cardiac index is 1.75 and } R = \frac{91.1}{1.75} \times 3 = 156$$

An independent estimate of the cardiac output by acetylene was precisely 2.95 liters per minute.

Respiratory estimates. The acetylene values with which comparison has been made have been obtained in the ordinary manner. Since Grollman's contention, that mixing in the lungs was inefficient in many subjects when lying down, was confirmed, the subjects were kept lying down for the estimation of oxygen consumption, and then were made to sit up rapidly to start the re-breathing procedure. Usually only two samples were drawn, but the whole procedure, including the determination of the oxygen consumption, was then repeated in duplicate, and the agreement between the two estimates was usually excellent. *Standing*: occasionally, at the end of the experiment, estimations of blood pressures, pulse wave

TABLE 1

Summary

Number of comparisons with acetylene on same day

Ten male subjects, 3 female subjects; ages 21 to 76; pulse rates 40 to 132; systolic pressures 90 to 205; and surface areas 1.35 to 1.89.

		NO.	MEAN DISCREPANCY (In per cent of acetylene value)
	Total number of comparisons	42	±11.9
	(Excluding doubtful values)	28	±6.2
Basal lying	(Including some doubtful values)	32	±12.5
	(Excluding doubtful values)	20	±5.9
Standing	(Including 1 doubtful value)	6	−10.5
	(Excluding doubtful value)	5	−7.6
Sitting	(Including 2 after meal)	3	±6.7
After meal	(Including 1 lying and 2 sitting and including 1 doubtful value)	3	±12.0

velocities, oxygen consumption, and the acetylene procedure were repeated with the subject standing; under these conditions the records obtained were necessarily fewer, and the errors greater. Except during such experiments where time was important, no circulatory data were obtained during the measurement of oxygen consumption, as breathing through valves was observed to alter the circulation considerably.

Results. Though only a few subjects have been used, they have been selected to include a wide range of ages and physique. The comparisons of the results of calculation with estimates by acetylene made on the same day are summarized in table 1. It will be seen that the average agreement is good. The results are summarized by individual subjects in

table 2. The numbers in brackets, which follow many of the subjects in table 2, refer to the numbers assigned to these same subjects in the papers by Doctors Starr, Gamble and Donal, and their associates (1934). Table 2 demonstrates that the equations are valid over the range of ages

TABLE 2

Summary: mean values of cardiac output for different subjects

SUBJECT, AGE, SEX, SURFACE AREA	BASAL-LYING VALUES CARDIAC INDICES: Acetylene No.	Acetylene Index	Ethyl iodide No.	Ethyl iodide Index	MEAN VALUES OF CARDIAC INDICES: Calc. No.	Calc. Index	Per cent dev. from acet.	OTHER CONDITIONS (INDIVIDUAL VALUES) CARDIAC INDICES: Condition	Acet.	Calc.	Per cent dev. from acet.
1. 21 M. 1.88 (230)	4	2.15 ±0.15	2	2.20 ±0.10	4	2.17 ±0.15	±7				
2. 22 M. 1.89 (226)	3	2.30 +0.10	1	1.90	1	2.60	+10				
3. 24 M. 1.59 (52)			4	3.02 ±0.17	2	2.55 ±0.25					
4. 25 F. 1.35 (229)	4	2.25 ±0.25	2	3.00 ±0.10	4	2.65 ±0.20	+24	Standing Sitting after meal	2.4 1.95	1.8 1.9	−25 −3
5. 28 F. 1.67 (152)			6	2.73 ±0.26	2	2.40 ±0.10		Lying after meal		3.0	
6. 30 M. 1.69 (223)	4	2.15 ±0.20	3	2.37 ±0.24	5	2.14 ±0.23	±8.5	Standing Standing	2.3 1.8	2.2 1.5	−4 −17
7. 32 F. 1.84	1	2.80	4	1.86 ±0.22	1	2.80	0				
8. 33 M. 1.66 (225)	5	2.12 ±0.13	2	2.25 ±0.05	4	2.22 ±0.18	±7	Standing	2.2	2.2	0
9. 43 M. 1.72 (224)	4	1.95 ±0.05	2	2.55 ±0.05	4	1.78 ±0.07	−9				
10. 48 M. 1.77 (220)	5	2.00 ±0.12	4	1.70 ±0.22	7	2.19 ±0.16	±9	Standing Standing	2.3 2.3	1.9 2.3	−17 0
11. 50 M. 1.78 (97)	1	(1.90)	1	(3.00)	1	2.70	(+42)	Sitting	2.0	1.9	−5
12. 63 M. 1.88	2	1.90 ±0.10			2	1.95 ±0.25	±8	Lying after meal	(2.6)	1.8	−21
13. 76 M. 1.62	2	1.85 ±0.05			2	1.95 ±0.05	+5				

and sizes tested. The somewhat greater discrepancy in the smallest subject is probably not significant, as other factors can explain it. In table 3, the mean values of the blood pressure data are given for the various subjects; F represents pulse rate, Sp, systolic pressure estimated by the recurrence of a peripheral pulse, S the systolic, Z the dicrotic, and D the

TABLE 3

Summary: Mean values of pressures, etc., for different subjects

SUB., AGE, SEX, HEIGHT (CM.), WEIGHT (KGM.)	CARDIAC INDICES		F	Sp	S	Z	D	M	M_s	M_d	PULSE WAVE VELOCITIES				S/C	R	CONDITIONS
	Acet.	Calc.									1	2	3	4			
1. M. 21 178 70.6	2.15	2.17	60.2	107.8	101.0	86.2	55.8	77.2	84.5	70.8	3.53	5.47	5.50	10.20	0.463	109.5	Basal—lying
2. M. 22 177 74.0	2.30	2.60	70.0	108.0	102.0	89.0	59.0	84.0	94.0	74.0	3.90	4.90	5.10	9.20	0.490	97.0	Basal—lying
		2.80	*88.0*	*124.0*	*116.0*	*108.0*	*80.0*	*97.0*	*102.0*	*93.0*	*2.80*	*5.90*	*5.50*	*14.60*	*0.470*	*104.0*	*Standing*
3. M. 24 169 52.0		2.30	63.0	94.0	86.0	80.0	56.0	74.0	81.0	68.0	3.30	4.40	5.50		0.420	96.0	Basal—lying
4. F. 25 146 46.0	2.25	2.65	81.8	97.0	91.2	79.5	58.5	75.7	80.8	68.8	3.20	4.95	5.57	9.47	0.570	87.0	"Basal"—lying; not relaxed
	2.40	*1.80*	*132.0*	*100.0*	*94.0*	*84.0*	*70.0*	*83.0*	*89.0*	*77.0*	*3.20*	*8.60*	*8.00*	*15.00*	*0.530*	*138.0*	*Standing*
	1.95	*1.90*	*101.5*	*100.0*	*97.5*	*89.5*	*72.0*	*87.0*	*90.5*	*81.0*	*3.60*	*6.70*	*7.25*	*11.05*	*0.590*	*137.5*	*Sitting after meal*
5. F. 28 168 58.8		2.40	69.0	100.5	93.5	75.5	57.0	76.0	83.5	65.5	2.95	6.75	6.05	7.10	0.545	95.0	Basal—lying
		3.00	*81.0*	*102.0*	*93.0*	*68.0*	*51.0*	*72.0*	*80.0*	*60.0*	*3.10*	*8.10*	*6.10*	*7.90*	*0.630*	*72.0*	*Lying after meal*
6. M. 30 167 62.3	2.15	2.14	63.2	114.4	107.2	91.4	67.2	86.8	96.2	79.4	3.12	7.49	6.08	8.60	0.454	124.0	Basal—lying
	2.05	*1.85*	*82.0*	*133.5*	*129.0*	*114.5*	*95.5*	*111.0*	*118.0*	*105.0*	*3.00*	*8.25*	*7.50*	*14.30*	*0.460*	*187.0*	*Standing*
7. F. 32 177 69.3	2.80	2.80	77.0	106.0	97.0	72.0	53.0	74.0	85.0	62.0	3.20	6.30	5.80	8.10	0.520	79.0	Basal—lying
8. M. 33 179 52.9	2.12	2.22	49.4	110.2	105.4	93.2	60.0	82.6	94.2	76.6	2.88	6.92	6.22		0.346	113.0	Basal—lying
	2.20	*2.20*	*66.0*	*135.0*	*132.0*	*114.0*	*93.0*	*109.0*	*118.0*	*104.0*	*2.40*	*8.90*	*7.20*		*0.370*	*149.0*	*Standing*
9. M. 43 170 63.5	1.95	1.78	44.2	101.8	96.8	90.5	64.5	80.8	86.8	77.5	2.70	6.65	6.50		0.322	138.0	Basal—lying
		1.70	*59.0*	*109.0*	*107.0*	*98.0*	*79.0*	*90.0*	*95.0*	*88.0*	*2.30*	*10.60*	*10.90*		*0.300*	*159.0*	*Standing*
10. M. 48 174 65.5	2.00	2.19	52.6	106.4	100.7	85.6	62.0	82.4	90.1	73.7	3.07	6.63	6.49		0.387	113.0	Basal—lying
	2.00	*1.80*	*61.0*	*118.3*	*114.3*	*96.0*	*78.7*	*93.0*	*103.5*	*88.0*	*3.05*	*7.92*	*6.92*		*0.372*	*160.0*	*Standing*
11. M. 50 169 69.0	(1.90)	2.70	50.0	148.0	147.0	125.0	73.0	111.0	136.0	99.0	3.50	8.80	8.20	14.80	0.320	123.0	Basal—lying
	2.00	*1.90*	*59.0*	*160.0*	*154.0*	*132.0*	*87.0*	*122.0*	*144.0*	*109.0*	*4.30*	*12.70*	*8.30*	*13.60*	*0.370*	*193.0*	*Sitting*
12. M. 63 181 69.0	1.90	1.95	53.0	99.5	94.5	80.5	55.5	74.5	83.5	68.0	3.80	8.40	6.40	11.30	0.415	117.5	Basal—lying
	(2.60)	*1.80*	*58.0*	*108.0*	*102.0*	*87.0*	*63.0*	*83.0*	*94.0*	*75.0*	*4.10*	*7.40*	*7.40*	*11.40*	*0.410*	*139.0*	*Lying after breakfast*
13. M. 76 162 59.5	1.85	1.95	47.0	202.0	171.0	149.0	70.5	118.0	133.0	110.5	5.60	11.70	7.40	13.70	0.350	182.0	Basal—lying
Means of subjects of less than 40 years age	2.29	2.41	68.9	104.7	97.9	83.3	58.3	78.8	87.4	70.6	3.26	5.90	5.73	8.78	0.476	100.1	Basal—lying
	2.15	*2.11*	*93.9*	*118.5*	*113.7*	*102.0*	*82.1*	*97.4*	*103.5*	*92.0*	*3.00*	*7.27*	*7.09*	*13.74*	*0.484*	*143.1*	*Standing or sitting*
Means of subjects 43 to 63 years of age	1.94	2.15	49.9	113.9	109.8	95.4	63.8	87.2	99.1	79.6	3.27	7.62	6.90	13.05	0.361	122.9	Basal—lying
	2.00	*1.80*	*59.7*	*129.1*	*125.1*	*108.7*	*81.6*	*101.7*	*114.2*	*95.0*	*3.22*	*10.41*	*8.71*	*13.60*	*0.347*	*170.7*	*Standing or sitting*

TABLE 4

Effect of standing

CONDITION	DATE	TEMP.	SUBJ.	CARDIAC INDICES: Acet.	CARDIAC INDICES: Ethyl Iodide	CARDIAC INDICES: Calc.	F	S	Z	D	PULSE WAVE VELOC. 1	PULSE WAVE VELOC. 2	PULSE WAVE VELOC. 3	PULSE WAVE VELOC. 4	R
Lying	June 16	18.3	10	—	—	2.3	57	88	74	52	3.0	7.0	6.2	—	89
Standing	June 16	18.3	10	—	—	1.6	72	109	91	78	3.0	8.2	6.8	—	170
Lying	June 23	21.7	10	—	2.0	2.3	53	99	84	59	2.8	6.3	7.2	—	101
Standing	June 23	21.7	10	—	—	1.4	66	116	90	75	3.4	8.7	7.4	—	195
Lying	Aug. 16*	22.2	10	(2.1)	—	2.2	50	105	90	64	3.5	6.5	6.1	—	113
Standing	Aug. 16	22.2	10	2.3	—	1.9	57	116	104	83	3.2	7.7	6.5	—	147
Lying	Aug. 22*	21.7	10	(1.9)	—	2.2	48	105	95	67	2.9	7.0	6.7	—	120
Standing	Aug. 22	21.7	10	2.3	—	2.3	49	116	99	75	2.6	7.1	7.0	—	127
Lying	Aug. 24*	21.9	6	(2.2)	—	2.0	60	106	93	65	3.3	7.9	6.0	—	133
Standing	Aug. 24	21.9	6	2.3	—	2.2	79	126	115	92	3.2	7.0	5.9	—	150
Lying	Aug. 25*	22.1	8	2.2	—	2.2	51	111	101	63	3.1	7.9	6.8	—	115
Standing	Aug. 25	22.1	8	2.2	—	2.2	66	132	114	93	2.4	8.9	7.2	—	149
Lying	Sept. 1*	20.5	9	(2.0)	—	1.7	41	96	91	61	2.8	5.5	6.3	—	138
Standing	Sept. 1	20.5	9	—	—	1.7	59	107	98	79	2.3	10.6	10.9	—	159
Lying	Sept. 13	16.9	9	1.9	2.55	1.8	52	90	84	65	2.5	7.0	6.5	—	129
Standing	Sept. 13	16.9	9	1.65	2.15	—	82	—	—	—	—	—	—	—	—
Lying	Sept. 20	Cool	10	2.2	1.5	—	56	—	—	—	—	—	—	—	—
Standing	Sept. 20	Cool	10	1.4	1.3	—	73	—	—	—	—	—	—	—	—
Lying	Oct. 5	Cool	8	2.25	2.25	2.5	49	103	95	63	2.6	6.6	6.0	—	103
Standing	Oct. 5	Cool	8	1.6	1.75	—	77	—	—	—	—	—	—	—	—
Lying	Oct. 9	Cool	2	2.2	1.95	—	72	—	—	—	—	—	—	—	—
Standing	Oct. 9	Cool	2	1.85	1.55	—	93	—	—	—	—	—	—	—	—
Lying	Oct. 26	17.3	6	1.8	2.55	1.8	67	116	96	75	3.1	7.8	6.3	8.6	152
Standing	Oct. 26	17.3	6	1.8	—	1.5	90	132	114	99	2.8	9.5	9.1	14.3	224
Lying	Nov. 1	20.5	2	2.35	—	2.6	70	102	89	59	3.9	4.9	5.1	9.2	97
Standing	Nov. 1	20.5	2	—	—	2.8	88	116	108	80	2.8	5.9	5.5	14.6	104
Lying	Nov. 7	21.7	4	—	(3.0)	2.6	86 to 92	90	76	57	3.1	6.6	7.2	9.7	87
Standing	Nov. 7	21.7	4	2.4	2.35	1.8	117 to 132	94	84	70	3.2	8.6	8.0	15.0	138

Mean changes on standing

	CARDIAC INDICES: Acet.	CARDIAC INDICES: Calc.	PULSE RATE	S	Z	D	PULSE WAVE VELOC. 1	PULSE WAVE VELOC. 2	PULSE WAVE VELOC. 3	R
On 5 starred days........	+0.18	0.00	+12	+15	+12	+20	−0.38	+1.3	+1.1	+23
On 9 other days..........	−0.41	−0.50	+22	+14	+16	+20	−0.14	+1.7	+0.96	+61

diastolic pressures by the lateral criteria; M, M_s, and M_d the mean pressures estimated for the cycle, for the s (nominal systolic) period (including the dicrotic notch), and for the d (nominal diastolic) period; the pulse wave velocities from heart to subclavian, subclavian to femoral, subclavian to brachial, and femoral to dorsalis are numbered 1 to 4; s/c is the ratio of the s period to that of the cycle as measured on the brachial pulse, and R the effective resistance calculated from the ratio of the mean pressure to the calculated cardiac index.

The accuracy obtained by calculation by the second equation given, which was used in the earlier experiments (since at this time measurements of the pulse wave velocity from the femoral to dorsalis pedis were not included), is for basal conditions, almost, but not quite, as good as that by the first. For conditions other than basal the more complete first equation seems to be definitely superior. Calculations can also be made, if desired, from the Sp estimate of systolic pressure, but no constants have been found which give as good agreement as is attainable by calculation from the S values.

The average effect of standing is shown in table 3. It may be noted that there is little indication of reduction of the cardiac output on standing, a result in agreement with Grollman (1930), and in disagreement with the data obtained by one of us, on himself (JCS., 1935). Since the data indicate a seasonal factor, so that subject 8 (JCS.) can give either result at different times, they merit more detailed treatment. In table 4 the experiments on standing are detailed in chronological order as obtained during a hot summer period in 1933. (The temperatures given in table 4 represent effective temperatures on the basic scale; the higher temperatures imply dry bulb temperatures of over 25° and wet bulb temperatures of over 21°C. On other days, not here listed, temperatures were considerably higher.) The results obtained in August and early September all show, both by acetylene and by calculation, no reduction of cardiac output on standing, a relatively slight increase in pulse rate (which is particularly shown in the data on individual subjects) and a rather definite reduction in the pulse wave velocity in the aortic arch. On the other hand, the data obtained in June, as the warm weather was commencing, and in September, during cooler weather, show a much greater change in pulse rate, and a reduction in cardiac output. In November, during a recurrence of moderately warm outside weather, with heated rooms, the data do not fall clearly in either group. Further work on the effect of standing on cardiac output will be published later by one of us (JCS.).

Discussion. Though the equations have been demonstrated to be fairly adequate for basal conditions, they are only to be so regarded if the observations are made with meticulous care, if the subject is in a relatively stable condition, so that records obtained over a relatively long period may

be justifiably averaged, and, even if these conditions are fulfilled, such calculations must not be carried over to conditions other than basal, until the empirical equations have been demonstrated to be valid for such conditions. It has to be remembered that the pressures determined from the brachial artery are assumed to be representative of those of the whole arterial tree, an assumption which is certainly only partially true in the horizontal (Bazett, 1924) and even less true in the vertical position. The agreement obtained between the two estimates with the subject standing was, therefore, better than was anticipated. The care necessary in obtaining the data may be realized from a consideration of table 3. The calculations are all dependent on the differences between the dicrotic wave and diastolic pressures, which average, in the young subjects, 24 mm. lying and 20 mm. standing; an error of 5 mm. in estimating this difference will by itself, therefore, cause an error of 20 to 25 per cent in the cardiac index, and such an error has to be avoided even though the two pressures sometimes show respiratory or other rhythmic variations of 5 to 10 mm. A series of blood pressure measurements, which can legitimately be averaged, is, therefore, essential.

When a subject shows a pulse rate that increases during the taking of the records, the slower pulse rates observed between records should be used for calculation, though even so the estimate is apt to be somewhat too high. Sometimes (though rarely under basal conditions) marked reflected waves can distort the brachial pulse; under such conditions pulse wave measurements should be made on the subclavian record, in spite of the difficulties introduced by respiratory movements.

As far as the size of the aorta is concerned, an estimate of the diameter from an orthodiagram might be expected to be more satisfactory than any theoretical curves, but the opposite has proved to be the case. Orthodiagraphic estimates of aortic diameter, even if correct, give only the external diameter at a certain point, while from the point of view of the calculations it is the average internal diameter and consequent contained volume that is important. In the older subjects tortuosity of the aorta may complicate the picture, and the orthodiagram indicated that such tortuosity was present in the aorta in subject 13. Any tortuosity must cause under-estimation of the contained volume, but also an under-estimate of the pulse wave velocity.

The importance attributed to the aortic arch as an elastic resevoir in the equations by the volumes and pulse wave velocities assigned to this section was somewhat surprising, though, perhaps, to be anticipated from Hürthle's data. The importance it attained in the equations was forced through the inadequacy of other equations to cope with the condition of a slow pulse, small pulse pressure and normal peripheral pulse wave velocities: it was substantiated on anatomical grounds later. Thus in subject

9, on one occasion, with a pulse rate of 45, a pulse pressure estimated as 30, and certainly less than 35, a normal cardiac output was attained, yet the pulse wave velocities from the subclavian to femoral and subclavian to brachial were 7.0 and 6.4 respectively, values very little below the averages of other subjects. A pulse wave velocity for the aorta below the normal value, such as was found (2.5), provided the only explanation of the surprisingly low pulse pressure. The pulse wave velocity in the aorta may fall (see table 4 for the effect of standing), even though both systolic and diastolic pressures rise. This must imply that the pulse wave velocity is not determined in these large vessels by the internal tension so much as by the condition of the muscular coat, which has often been considered a negligible factor. This implication has been abundantly supported by later data (Bazett, Scott, Maxfield and Blithe, 1935).

The Broemser and Ranke equation has not been tested adequately and cannot be, in that the criteria they use for pressure readings are different. We do not believe their criteria justifiable, since, in case of difficulty, the lateral systolic pressure is taken as the pressure at which a dicrotic wave re-appears in the radial pulse (Broemser and Ranke, 1933). However, applying their equation to our data as far as possible, the cardiac index calculated may be occasionally actually improved (e.g., in subject 4, lying, for Nov. 7, table 4, the index calculated by their equation is 2.3), but is also often absurd (e.g., the index calculated for the athletic subject 9, under basal conditions, from the data quoted previously when the pulse rate was 45 and the pulse pressure 30—is 0.8, and that for subject 13 from the data of table 3, is from 2.8 to 3.8 (according to the interpretation of the subclavian pulse).

From the apex beat records data were also obtained on the duration of the isometric contraction period and of mechanical systole. If the duration of systole be expressed in relation to the square root of the cycle (Bazett, 1920) according to an equation of the type:

$$\text{Systole} = M\sqrt{\text{cycle}}$$

the values of M were relatively constant for any one subject under standard conditions, and shorter in the standing than in other positions. As a rule, changes in M were in the same direction as changes in stroke volume. From the values given in the tables the work performed by the heart may be calculated with an accuracy greater than that obtained by the ordinary methods, since M_s represents approximately the resistance to be overcome by the heart in systole. The estimate of the area of the ascending aorta combined with the duration of systole and the length of the isometric period allows the mean velocity during expulsion to be estimated, though any integration of the curve is still impossible. The average values in seconds for the isometric period for the thirteen subjects under basal

conditions was 0.042 (extremes 0.030 and 0.056); and for M: 0.395 (extremes 0.361 and 0.437). For 7 subjects standing, the mean values were for the isometric period: 0.048 and for M: 0.365.

Using these values and the data of table 3, the work per beat of the left ventricle, including an estimate of that contributing to kinetic energy, was calculated for the subjects also utilized by Starr, Donal and their associates (1934). The work so calculated was about 5 per cent greater than they calculated; some 2.8 per cent of the total work is estimated as kinetic energy under basal conditions for the younger subjects, but only 0.8 and 0.6 per cent for the two oldest subjects. Owing to the impossibility of integrating curves, the kinetic energy is, no doubt, under-estimated, but the difference with age is significant. When the total work, so calculated from mean values, is correlated with the cardiac silhouette as suggested by Starr and his co-workers (1934), the degree of scatter is reduced by nearly 50 per cent in these subjects as compared with the data calculated by them from individual values and auscultatory pressures.

No claim is made that the method of determining the cardiac output from the blood pressures is simpler, less laborious, or more accurate than the respiratory methods; it is certainly more laborious, and accuracy is hard to attain. On the other hand, it appears to be the sole method applicable to ill patients; respiratory diseases do not interfere with it; it requires no coöperation by the subject; if applied successfully, it gives information not only as to the cardiac output, but also as to the condition of the large vessels, of the effective resistance of the arterioles, and a measure of cardiac work. It should, therefore, prove useful for clinical investigation, if adequately controlled, when possible, by respiratory methods.[2]

SUMMARY

1. If blood pressure is taken by the method previously described, the lateral pulse pressure is found to be related to the stroke volume, if the form of the pulse curve is taken into consideration, and if the distensibilities of the large vessels are estimated by determining the pulse wave velocities from the heart to the subclavian, subclavian to femoral, subclavian to brachial, and femoral to dorsalis pedis. In making such calculations, the size of the vessels in relation not only to the size but also to the age of the individual must be considered. Curves are given which may be used to predict this size.

2. Equations are given by which these calculations may be made; they depend primarily on the deduction of the outflow from the arterial tree in diastole, from the fall of pressure from the dicrotic wave to the diastolic level, and of the systolic outflow from the relative durations of, and rela-

[2] Experiments are in progress to test the equations under conditions of digestion of meals, and exposure to warmth and cold.

tive pressures in, systole and diastole. The constants used have mainly an empirical foundation based on comparison of such calculations with estimates by the acetylene method. A total of 42 such comparisons is reported with a mean discrepancy of ± 11.9 per cent of the acetylene value.

3. Most of the values reported were obtained with the subject basal, and lying down; a few were obtained during standing or in other conditions. The values for cardiac output obtained during standing sometimes indicated no reduction below those observed with the subjects lying down; at other times they indicated a considerable reduction; the presence of a seasonal variation was indicated.

We would like to thank Doctors I. Starr and Gamble and their associates for their free coöperation in this work, and for the examination of many of the subjects by their modified ethyl iodide method; also Dr. A. Grollman for advice in the use of his method and Dr. A. Margolies for examining many of the subjects with the orthodiagram.

APPENDIX

On the size and distensibility of the aorta in man, and the effect of age upon them. The calculation of the stroke volume from the pulse pressure by empirical equations required the prediction of the internal cross-sectional area of the aorta. It is proposed here to mention briefly the evidence on which these values were based, and to discuss their relationship to observed pulse wave velocities.

The *size of the aorta* in man has been often measured; the articles by Thoma (1882), Suter (1897), and Kani (1910) may be mentioned, as well as estimates of the size in life by Roentgen rays by Smith (1920), and by Vacquez and Bordet (1928). Owing to the large number of cases examined (2,719) Suter's data are probably the most important; his subjects are grouped by age, height and sex, but from the average weight of the different groups the mean surface areas for the groups can be approximately deduced. Dreyer, Ray and Walker (1912) showed that there is a correlation of aortic cross-sectional area with surface area. Consequently, from the data given by Suter for the circumference of the undistended aorta the respective cross-sectional areas have been calculated and plotted against the surface areas of the appropriate groups without regard to sex. The graphs so obtained are shown in figure 4; a linear relationship to surface area appears in each age group, but the slopes of the lines vary with age, for the increase in the size of the aorta with age is more marked the larger the individual (compare the data of Dublin (1930) on the effect of size of the individual on the incidence of arterio-sclerosis).

Not only does the ascending aorta increase in size with age, but also the other large vessels. This may be seen in the data of Kani plotted in figure 5. It is obvious that the smaller vessels such as the carotids and

renals do not share in this increase. Kani's data also demonstrate a change in the ratio of the thickness of the vessel wall to that of the internal

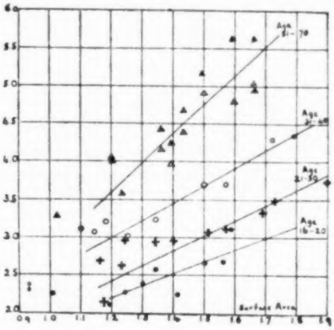

Fig. 4

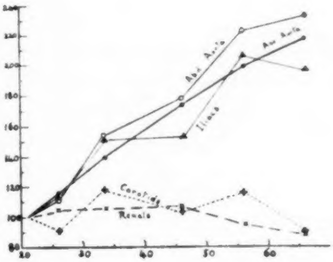

Fig. 5

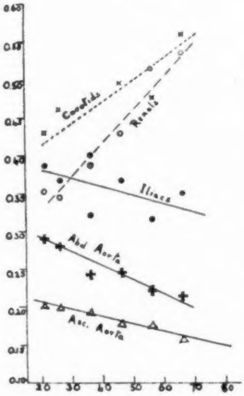

Fig. 6

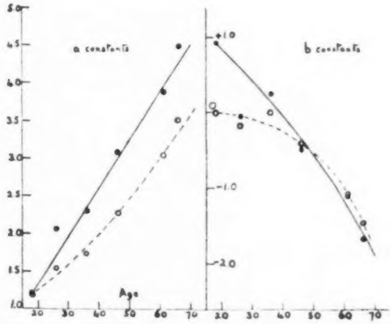

Fig. 7

Fig. 4. Cross-sectional areas (external) of aorta plotted against surface area for different age groups (Suter's data). For the uppermost line the open triangles represent values from subjects of age 51 to 60, the solid triangles those of age 61 to 70.

Fig. 5. The percentage increase in cross-sectional area of various vessels as ordinates plotted against age in years as abscissae (Kani's data).

Fig. 6. Ratio of the thickness of the vessel wall to its internal radius as ordinates plotted against age in years as abscissae for various vessels (Kani's data).

Fig. 7. Constants from which the external and internal cross-sectional area of the ascending aorta may be calculated by the equations given in the text for subjects of different age (abscissae). The heavy lines and solid circles indicate the values for the external cross-sectional area, the dotted lines and open circles those for the internal.

radius with age (though this statement is at variance with Frank's results (1927)). Kani's data are shown graphically in figure 6, and again a strong contrast may be seen between the smaller vessels, which show an increase

in the thickness of the wall with no increase in lumen, and the larger vessels, in which both the lumen and the thickness of the vessel wall increase, but the former at a greater rate than the latter.

The data of figure 4 may be represented by equations of the type

$$A = a\,S + b$$

where A is the external cross-sectional area of the aorta in square centimeters, S is the subject's surface area in square meters, and a and b are constants which vary with age. The values of these constants a and b for the curves of figure 4 are shown in figure 7. By applying the data

TABLE 5

Diameter of ascending aorta according to age and size, from graphs of Suter's data

AGE	SURFACE AREA OF SUBJECTS IN SQUARE METERS						AGE RANGE (VACQUEZ AND BORDET)
	1.2	1.4	1.6	1.8	2.0	2.2	
18	1.75	1.83	1.92	2.00	2.07	2.14	
20	1.75	1.83	1.92	2.00	2.08	2.15	2.0
25	1 82	1.92	2.03	2.13	2.22	2.32	
30	1.87	2.00	2.12	2.24	2.34	2.45	
35	1.93	2.07	2.21	2.33	2.45	2.59	2.0 to 2.5
40	1.98	2.14	2.30	2.44	2.57	2.69	
45	2.00	2.17	2.34	2.49	2.64	2.77	2.5 to 2.8
50	2.02	2.22	2.40	2.56	2.72	2.86	
55	2.08	2.29	2.48	2.67	2.82	2.98	2.5 to 3.0
60	2.10	2.33	2.53	2.72	2.89	3.06	
65	2.12	2.36	2.57	2.77	2.95	3.13	3.0 to 3.5
70	2.13	2.38	2.62	2.82	3.02	3.20	
75	2.12	2.39	2.63	2.86	3.07	3.26	

of Kani shown in figure 6, the thickness of the wall can be estimated, and so the internal cross-sectional area. This too may be represented by equations of the same type, and the constants a and b may similarly be read from figure 7 (dotted lines). That the values so determined agree reasonably well with figures determined in life by Roentgen rays is indicated in table 5, where the values calculated for the external diameter of the ascending aorta from the values of figure 7 are given for subjects of varying surface area for comparison with Vacquez and Bordet's estimates of the normal ranges. The calculated values for subjects of average size are, for the most part, slightly below the orthodiagraphic standards; as

the autopsy measurements were made without distention, this underestimation is not surprising.

The volume of the aorta in man has been measured by Hwiliwitzkaja (1926), but the values obtained appear inconsistent. A single aorta has, consequently, been measured for comparison with the curves which represent the cross-sectional area (other autopsies have so far not been available owing to the interference of the procedure used with the ordinary examination and embalming).

The subject, a negro aged 60, of height 169 cm., weight 63 kilo, and of surface area 1.7 sq. m., died following an intracranial operation. There was a history of syphilis, and the vessels were large and somewhat tortuous. The aorta was injected in situ with plaster of paris at a pressure of 100 mm. Hg. The aorta and its branches were divided into sections for comparison with the usual pulse wave velocity measurements. The volumes were: for the first section to a distance of 20 cm. from the heart along the outer curvatures of the vessels, 80.5 cc.; for the 2nd section, including the rest of the thoracic aorta, abdominal aorta, iliacs and the commencement of large vessels such as the coeliac axis, 118 cc. The total contents were, therefore, 198.5 cc.; this was considered to be an over-estimate for the normal subject owing to the tortuosity of the vessels, a tortuosity which became much more marked when the vessels were distended under pressure. The internal cross-sectional area of the aorta just above the valves was 6.6 sq. cm., and just before the origin of the branches 5.3 sq. cm. By the constants of fig. 7 the external cross-sectional areas of the aorta just before the origin of the branches is calculated as 5.45 sq. cm. with an internal area of 4.15 sq. cm. The graphs, as expected, give too low an estimate of the area for the aorta distended at its working pressure. The volumes calculated by the equations given in the earlier paper and used for estimation of stroke volume are 84 cc. and 105, with a total volume of 189 cc.

This single experiment demonstrates that the values assumed for cross-sectional area and volumes are not far removed from those actually found. Constants which gave better agreement with the observed values have also been used for calculation, but proved not so satisfactory as those ultimately adopted. As has been explained, it is the effective, rather than the true volumes, that are required for such calculations.

The distensibility of the aorta. The data imply that the size of the aorta increases with age, that the change begins immediately adult life is reached, and that in this there is a parallel to the changes in the modulus of elasticity described by Hochrein (1926), and Frank (1927), but that no increase in pulse wave velocity is observed in the first section of the aorta until after 40 years of age; in fact, if anything, the velocity is decreased. On the other hand, in the 2nd section of the aorta (subclavian to femoral) there is evidence of an age increase in pulse wave velocity even at ages below 40 in agreement with the statistical analysis of the voluminous data presented by Hallock (1934), but the changes before the age of 40 are slight, and are readily masked by physiological variations. It would appear, therefore, that changes in size of the aorta in the earlier

decades are able to compensate for the increase in the modulus of elasticity, for the pulse wave velocity may also be represented by the equation:

$$v = \sqrt{\frac{ED}{2er}} \qquad \text{(Bramwell and Hill, 1922)}$$

where v is the pulse wave velocity, E the transverse elasticity coefficient, D the thickness of the vessel wall, r its radius, and e the density of the fluid. It is, therefore, possible for a decrease in the ratio D/r to compensate for an increase in E. But the net result of all these changes is, with increasing age, a gradual shift to the central vessels of the reservoir action of the arterial tree, so that the large central vessels accommodate a larger proportion of the total stroke volume. This is in line with the discussion of the size of the aorta by Hess (1927), who points out that in the lower age ranges the aortic cross-sectional area is less than, and at the higher age ranges, is more than the total of its large branches.

Though the evidence is indirect, we believe that the increase in the size of the aorta with age is a change which compensates for loss of distensibility, in that the stroke volume forms a smaller percentage of the total volume and so creates a relatively smaller pressure change, while pressure changes are more efficiently transmitted into a stretching transverse tension of the wall; these relationships are expressed in Bramwell and Hill's (1922) and in Frank's (1920) equations:

$$dV = \frac{2\pi r^3 dp}{ED}$$

$$\sigma_t = \frac{pr}{D}$$

where $d\,V$ is the absolute change in volume, p is the pressure, σ_t is the transverse tension per unit cross-sectional area of the wall, and the other symbols are as before.

Tortuosity of the larger vessels may be considered as a similar compensatory change, in which the lengthening is in the long axis, a change which not only has similar effects, but which also, in the smaller vessels, as shown by Müller (1924), lowers the resistance to flow by the increased stretch which results from centrifugal forces.

SUMMARY OF APPENDIX

The data, on which curves representing the size of the aorta in man at different ages have been based, are discussed in relation to the pulse wave velocities observed over a wide age range. The marked enlargement of the aorta is shown to be probably compensatory to the changes in the modulus of elasticity, and to be the only possible explanation of the con-

tinued capacity of the system to act as an efficient reservoir. Tortuosity is considered to be merely an indication of an enlargement in the longitudinal axis, which in the large vessels, at any rate, may have a similar compensatory action. With increasing age the reservoir action of the large central vessels becomes proportionately more important than that of the peripheral vessels.

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THE RELATION OF THE SUPRARENAL CORTICAL HORMONE TO NITROGEN METABOLISM IN EXPERIMENTAL HYPERTHYROIDISM[1]

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Forty-two years ago it was demonstrated that the administration of thyroid gland to a normal animal or to a human being produced a negative nitrogen balance. It also has been shown that thyroxin produces the same effect (9). The degree and extent of the negative nitrogen balance produced by thyroxin is variable, but the cause of this variation is not understood. In the presentation of these results, the effect of a new factor on the amount of nitrogen that is lost will be demonstrated.

LITERATURE. That there may be some relationship between the action of the hormone of the suprarenal cortex and that of the thyroid gland is suggested by a review of the literature. Following the administration of thyroid gland to an experimental animal an increase in the size and weight of the suprarenal glands has been observed (8), (16). This hypertrophy was interpreted as evidence of an increase in physiologic activity. Enlargement of the suprarenal glands has been observed occasionally after atrophy of the thyroid gland or after its complete removal (2), (14). Gley (2) said that, in the glands which he studied, this enlargement proved histologically to be the result of fatty infiltration of the cortical cells and that in such instances the enlargement was by no means a sign of hyperfunction.

The relation of the thyroid gland to the suprarenal glands, so far as their influence on the basal metabolic rate is concerned, may be summarized as follows: Marine and Baumann (11) and others (1), (17) have demonstrated that complete removal or destruction of all cortical tissue results in a significant decrease in the basal metabolic rate. Severe, sufficient, sublethal injury to the cortical tissue of experimental animals may result in an increase in the basal metabolic rate, which is of variable duration (11), (12), (15). Marine and Baumann have demonstrated that this is the result of the presence of the thyroid gland because its removal

[1] Abridgment of thesis submitted to the Faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Medicine.

prevents this reaction. Injections of cortical hormone of proved potency have not produced any change in the basal metabolism of normal animals (4), (17). The effect of the administration of cortical hormone on the lowered metabolic rate of animals which have acute suprarenal deficiency has been an elevation of the metabolic rate to or above the normal basal figures (6), (17). The effect on the lowered basal metabolic rate of patients who have Addison's disease agrees with the results obtained with animals (3). When no increase has been observed (7), it is probable that sufficient cortical hormone had not been administered.

The relation of the suprarenal cortical hormone to nitrogen metabolism may be summarized as follows: The excretion of nitrogen in the urine of animals which have acute suprarenal deficiency is decreased (5), (13) presumably because of the associated impairment of renal function. The nitrogen balance of patients suffering from Addison's disease has been found to be negative when they receive inadequate, and positive when they receive adequate, quantities of cortical hormone (3).

METHODS. In order to determine the interrelation between thyroxin and the cortical hormone, studies on the nitrogen balance have been carried out on fully grown, normal, unilaterally and bilaterally suprarenalectomized dogs to which were administered intravenously, at intervals of one to five weeks, two 10 mgm. doses of thyroxin on consecutive days. Variable doses of cortical hormone[2] were administered to these animals to determine whether or not the reaction to thyroxin could be modified, both as to the systemic effect and the effect on nitrogen metabolism. The suprarenal glands were removed in two stages, with the animal under ether anesthesia and using surgical technic in all cases.

The diet used in these experiments consisted of lean horse meat 44 per cent, cracker meal 44 per cent, lard 6 per cent, and bone ash 6 per cent. To 14 kgm. of this diet were added four one-pound cans of tomatoes and sufficient sodium chloride to insure a salt content of at least 1 per cent. The daily ration allotted each animal was approximately twice its basal requirement. All the diet was consumed by a certain time each day, so that all the nitrogen excreted as a result of digestion of the food for that day would appear in the urine of the same day.

The urine, feces, and hair were collected daily. The urine was collected with unusual precautions in order to obviate loss of nitrogen. Each animal was catheterized at the same time every day. The total nitrogen of each twenty-four hour specimen of urine was determined by the Kjeldahl method in duplicate. The hair and feces were collected for periods of one to two weeks and then were analyzed for total content of nitrogen,

[2] The cortical hormone was prepared in the laboratory of The Mayo Foundation essentially by the method outlined in the article appearing in Transactions of the Association of American Physicians **69**: 147, 1934.

by the Kjeldahl method. The percentage of nitrogen was determined for each new batch of diet by the Kjeldahl method. The blood urea was estimated by the van Slyke and Cullen modification of the Marshall method, as further modified at the Mayo Clinic.

Results. The results of this study are illustrated graphically (figs. 1 to 6). It is obvious that in a study of this kind, constant experimental conditions must be maintained for weeks at a time. Many factors arose in the course of the present experiments that invalidated the results of particular tests, such as failure of an animal to consume all its food, vomiting of food, infection and fever, and the loss of any part of the animal's urine. Accordingly, only the results of tests in which constant experimental conditions were maintained throughout the period required for the test were recorded in the charts. A word of explanation is necessary relevant to the portion of each chart dealing with the nitrogen balance work. The figures on the ordinate represent grams of nitrogen. The daily intake of nitrogen is shown by the solid black line *a*, the level of which is practically constant in all experiments with each animal. The total daily excretion of nitrogen, which is the sum of the quantity present in the urine, feces, and hair, is represented by the solid black line *b*. The daily excretion of nitrogen in the urine is shown by the broken line *c*. The loss of nitrogen in the feces and hair is shown by the broken line *d*. The consecutive days on which the two 10 mgm. doses of thyroxin were administered are indicated by arrows. The remainder of each chart is self-explanatory. One should observe that retention of nitrogen in any experiment was accompanied by a proportionate gain in weight.

A dog with one suprarenal gland intact is indistinguishable from a normal dog. The first administration of thyroxin to a unilaterally suprarenalectomized female dog (exp. 1 in protocols) produced a negative nitrogen balance, during which a total loss of 5 grams of nitrogen occurred (fig. 1). In the subsequent four days, the animal retained 4 grams of nitrogen. During the second test, 4 grams of nitrogen were lost. This was followed by a retention of 11 grams of nitrogen and by a proportionate gain in weight, so that at the beginning of the third test the quantity of protein in the dog was greater than in either of the two previous tests. When the animal again received two injections of the same amount of thyroxin, but with a daily dose of 30 cc. of cortical hormone, which was started two days before thyroxin was administered, only 2 grams of nitrogen were lost. It should be noted also that the increase in pulse rate was less marked and of shorter duration than in the two previous instances. No significant changes were noted in the value of blood urea. It should be emphasized that the only way in which experimental conditions differ in these three tests is that the animal received cortical hormone in the third test. Thus, the breakdown of protein was reduced when cortical

hormone was administered. This result indicated that the cortical hormone exerts a sparing action against the effect of thyroxin on nitrogen metabolism

Throughout each series of tests on any bilaterally suprarenalectomized dog, the animal received a quantum of the same solution of cortical hormone which was varied in different tests and which is represented graphi-

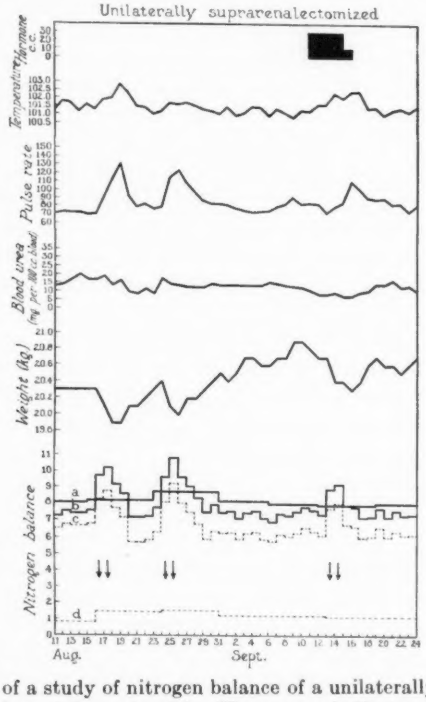

Fig. 1. Results of a study of nitrogen balance of a unilaterally suprarenalectomized female dog (exp. 1 in protocols). The arrows indicate consecutive days on which two 10 mgm. doses of thyroxin were administered. *a*, daily intake of nitrogen in grams; *b*, total daily excretion of nitrogen in grams; *c*, daily excretion of nitrogen in urine, in grams; *d*, daily loss of nitrogen in feces and hair, in grams. The significance of arrows and letters is the same in other figures.

cally by the number of shaded squares. In the first test with a bilaterally suprarenalectomized male dog (exp. 2 in protocols) while the animal received 10 cc. of cortical hormone daily, which was adequate for maintenance, thyroxin was administered, with the result that a rather prolonged negative nitrogen balance was produced, in which 6 grams of nitrogen were lost (fig. 2). Following this result, the nitrogen balance became positive and 15 grams of nitrogen were retained. Associated with

this retention of nitrogen was a proportionate gain in weight. When the same amount of thyroxin was injected again, while the animal received 30 cc. of cortical hormone daily, the nitrogen balance was maintained positive despite the fact that a slight increase in excretion of nitrogen occurred. This result indicates a sparing action of the cortical hormone against the effect of thyroxin on nitrogen metabolism.

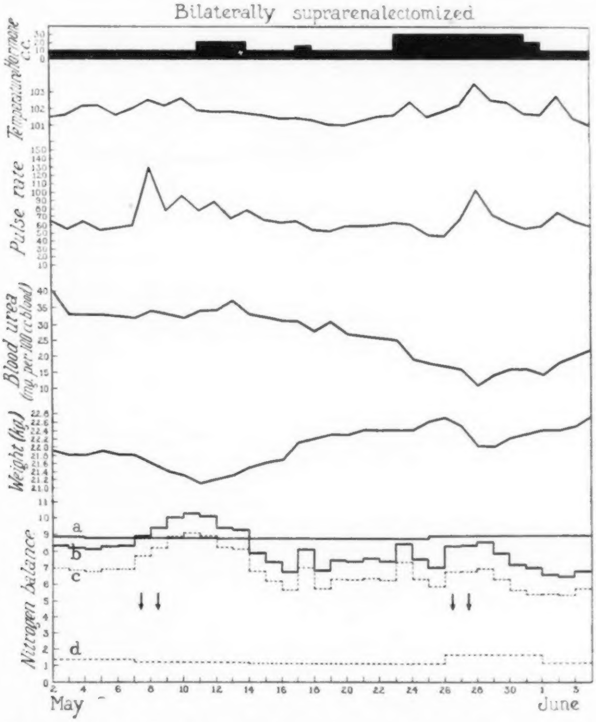

Fig. 2. Results of a study of nitrogen balance of a bilaterally suprarenalectomized male dog (exp. 2 in protocols).

In the first test with a unilaterally suprarenalectomized female dog (exp. 3 in protocols), the administration of thyroxin resulted in a negative nitrogen balance in which 3.5 grams of nitrogen were lost (fig. 3). This was followed by retention of 12 grams of nitrogen and a proportionate gain in weight. When the same doses of thyroxin were injected the second time, a total loss of 5 grams of nitrogen occurred. In the following twelve days, 9 grams of nitrogen were retained and associated with this retention of nitrogen was a proportionate gain in weight. After the animal had

received 20 cc. of cortical hormone daily for two days and while still receiving this amount, the same doses of thyroxin were administered again, but only 0.2 gram of nitrogen was lost. In the subsequent seventeen days, 10.5 grams of nitrogen were retained. In the last test, while no cortical hormone was given, the same doses of thyroxin were repeated and 4 grams of nitrogen were lost. In the third test, while the animal received large amounts of cortical hormone, the level of excretion of nitrogen was

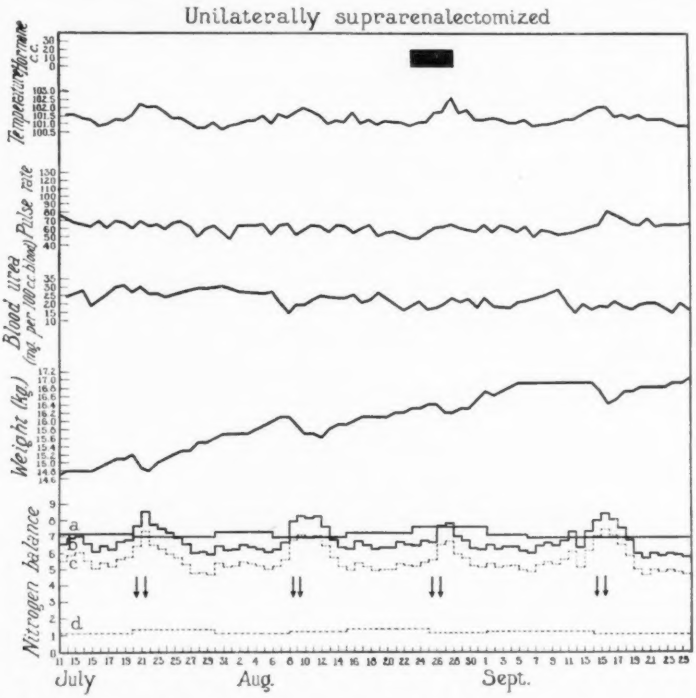

Fig. 3. Results of a study of nitrogen balance of a unilaterally suprarenalectomized female dog (exp. 3 in protocols).

not altered on the first day of the administration of thyroxin; whereas, on the same day in each of the other three tests, the total output of nitrogen exceeded the intake. No significant alteration in the pulse rate or of the level of blood urea occurred in this dog.

The remaining suprarenal gland of the same animal was removed. While 3 cc. of cortical hormone were administered daily, thyroxin was injected as described. This produced a negative nitrogen balance in which 4.5 grams of nitrogen were lost (fig. 4). Then the nitrogen balance be-

came positive, and during the next eight days 4.5 grams of nitrogen were retained. When the same doses of thyroxin were repeated, after the animal had received 20 cc. of cortical hormone daily for six days, and while still receiving this same amount, only 1.5 grams of nitrogen were lost. This was followed by retention of 12 grams of nitrogen and a very rapid gain in weight. When the same doses of thyroxin were repeated, while the animal received only 1 cc. of cortical hormone daily, a prolonged negative nitrogen balance resulted, in which 7.5 grams of nitrogen were lost, and there was a pronounced systemic effect as evidenced by the rather

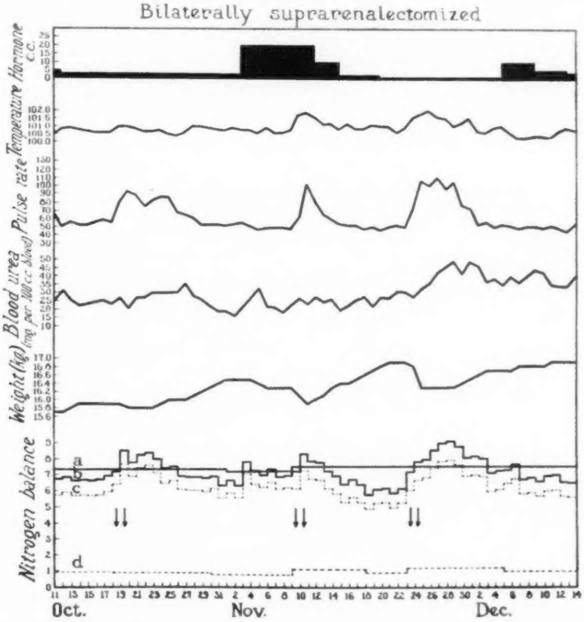

Fig. 4. Results of a study of nitrogen balance after removal of the remaining suprarenal gland from the female dog referred to in figure 3 (exp. 3 in protocols).

marked and prolonged elevation of the pulse rate and increased concentration of blood urea. The systemic effect is in contrast to the transient elevation of the pulse rate and the absence of any elevation of blood urea in the second test, while large amounts of cortical hormone were given.

The breakdown of protein was reduced as the amount of cortical hormone administered was increased. With the largest amount of cortical hormone, only a fifth as much nitrogen was lost as when the smallest amount was administered. The rapidity with which nitrogen is retained in the presence of large amounts of cortical hormone is strikingly demon-

strated in the second test. Twelve grams of nitrogen were retained in the ten days of recovery following this test. Only 4.5 grams of nitrogen were retained in the eight days of recovery after the first test. It is difficult to understand why nitrogen was not retained in the six days preceding the second test while the animal was receiving large amounts of cortical hormone, unless the doses of cortical hormone administered were so large as to exert some toxic effect.

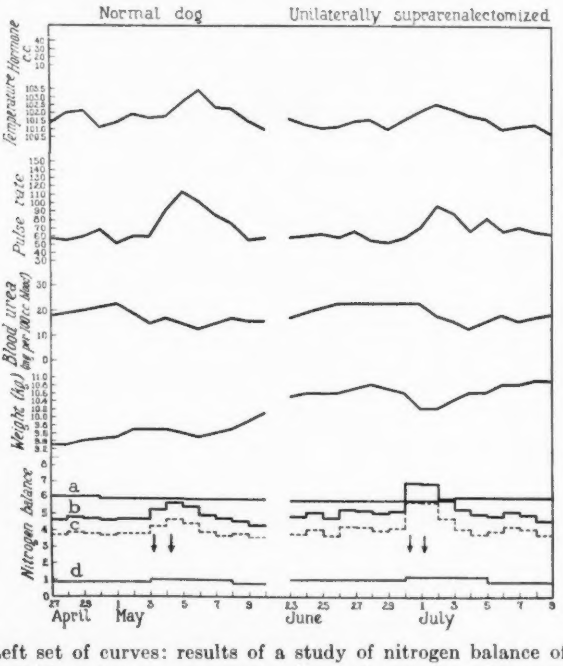

Fig. 5. Left set of curves: results of a study of nitrogen balance of a normal female dog. Right set of curves: results of a similar study on the same dog after right suprarenalectomy (exp. 4 in protocols).

Studies were made with a normal female dog (exp. 4 in protocols) and were repeated after the right suprarenal gland had been removed (fig. 5). In the period preliminary to the injections of thyroxin, 8 grams of nitrogen were retained. Following the administration of thyroxin, as described, the nitrogen balance remained distinctly positive, although a slight increase in the excretion of urinary nitrogen occurred. When the right suprarenal gland had been removed and the test was repeated, the nitrogen balance was negative for two days, during which time 2.5 grams of nitrogen were lost.

The remaining suprarenal gland then was removed. Subsequent to the administration of thyroxin, while receiving 2 to 4 cc. of cortical hormone daily, the animal lost 3 grams of nitrogen (fig. 6). During the succeeding eighteen days, the animal remained in nitrogen equilibrium. Nitrogen was not retained until the dose of cortical hormone had been increased. The nitrogen balance then gradually became positive, and at the start of the second test a total of 10 grams of nitrogen had been retained. When the same doses of thyroxin were injected again, after the

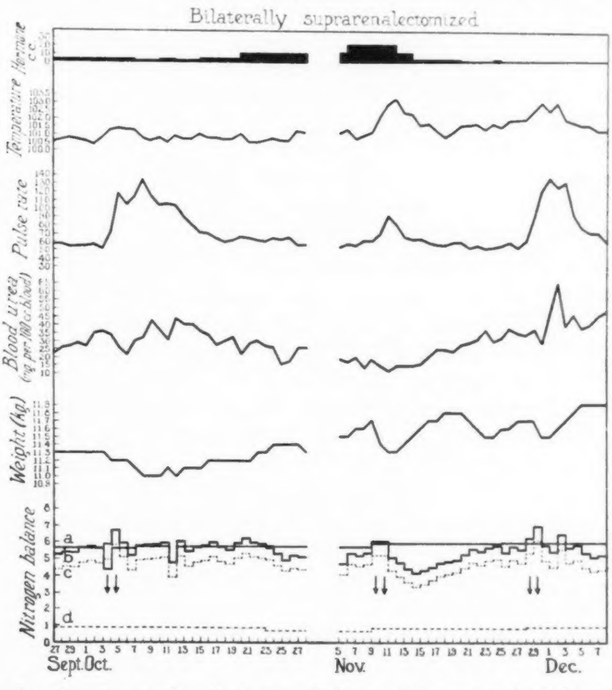

Fig. 6. Results of a study of nitrogen balance after removal of the remaining (left) suprarenal gland from the dog referred to in figure 5 (exp. 4 in protocols).

animal had received 20 cc. of cortical hormone daily for three days and while still receiving this quantity, practically no nitrogen was lost. In the seventeen subsequent days, 14 grams of nitrogen were retained; most of this retention occurred in the first twelve days. Following the administration of the same doses of thyroxin in the third test, while the animal was receiving 1 cc. of cortical hormone daily, the nitrogen balance became negative and 1.5 grams of nitrogen were lost.

In the last test, while the animal received a small amount of cortical

hormone, elevation of pulse rate was pronounced and prolonged; elevation of blood urea was significant; and associated with this was a diminution in the quantity of nitrogen excreted in the urine. This indicates suprarenal deficiency and associated impairment of renal function. The dose of cortical hormone was maintained constant throughout this period, and as the effect of thyroxin disappeared, the amount of hormone administered again became adequate and the level of the blood urea gradually declined. These observations may be contrasted with the results in the second test, while the animal received large amounts of cortical hormone. An insignificant elevation in the pulse rate occurred, and a striking depression of the level of blood urea resulted. The lessened systemic effect is striking. The rapidity with which nitrogen is retained in the presence of large amounts of cortical hormone is demonstrated again in the second test. In seventeen days, 14 grams of nitrogen were retained; most of this retention occurred in the first twelve days. This may be contrasted with the absence of any retention of nitrogen in the first test for eighteen days after the second injection of thyroxin. Retention of nitrogen occurred only after the daily dose of cortical hormone had been increased.

COMMENT. *Effect of thyroxin on the maintenance requirement of cortical hormone.* The influence of the cortical hormone on the physiologic response to thyroxin has been discussed. A word may be said concerning the effect of thyroxin on the maintenance requirement of the cortical hormone. If a suprarenalectomized animal which is receiving a small but adequate amount of cortical hormone be given an injection of thyroxin, a condition of profound suprarenal deficiency is produced, which may be rapidly fatal. The marked systemic effect produced by this procedure, together with the elevation of the blood urea, has been pointed out. Two animals were lost in this way (exp. 5 and 6 in protocols). Both of them appeared to be in good condition up to within eight or nine hours before death, when they collapsed and death ensued rapidly.

The work of Zwemer (18) is interesting in this connection. His observations were made while studying means of prolonging life after bilateral suprarenalectomy of cats. The suprarenal glands were removed in two stages. With the thyroid gland intact, the average time of survival after the second operation was fifty-three hours. If, however, the thyroid gland had been removed previously, the average time of survival after the second operation was 200 hours. When previously thyroidectomized animals were given 0.25 gram of desiccated thyroid substance daily, between the two operations, the average time of survival after the second operation was only eighteen hours. Clinically, administration of thyroxin to patients suffering from Addison's disease is attended by grave danger.

Relation of the thymus to the results obtained. The enlargement of the thymus observed in animals which have survived for a considerable time

the removal of both suprarenal glands is striking. An adult male police dog (exp. 5 in protocols) received cortical hormone for sixteen and a half months after the second suprarenal gland had been removed. At necropsy, the thymus was found to weigh 80 grams. The body weight of the animal at death was 35.6 kgm. An adult female shepherd dog (exp. 4 in protocols) was maintained with cortical hormone for more than three months after the second operation. At necropsy, the thymus was found to weigh 45 grams. The weight of the dog at death was 11.7 kgm. An adult female hound (exp. 1 in protocols) was treated with cortical hormone for more than four months after the second suprarenal gland had been removed and at necropsy the thymus was found to weigh 30 grams. An adult female shepherd dog which also was subjected to bilateral suprarenalectomy (exp. 3 in protocols), received cortical hormone for more than seven months and at necropsy the thymus was found to weigh 32 grams. In another case in which bilateral suprarenalectomy was performed, the animal was treated with cortical hormone for about two months (exp. 6 in protocols) and the thymus was found at necropsy to be enlarged greatly. Marine (10) and Swingle and Pfiffner (17) have made similar observations with suprarenalectomized animals.

The explanation of thymic hyperplasia of suprarenalectomized animals is not apparent. The fact that thymic hyperplasia occurs frequently in cases in which patients have exophthalmic goiter is well known. With the doses of thyroxin employed, it was not possible to produce a marked negative nitrogen balance in two of the dogs which had a large thymus (exp. 4 and 5 in protocols), even while small doses of cortical hormone were administered. It is possible that the hyperplastic thymus exerted an influence on the nitrogen metabolism.

Explanation of failure of others to obtain an increased excretion of nitrogen with suprarenalectomized dogs. When a bilaterally suprarenalectomized animal has acute suprarenal deficiency, the renal function is impaired, the blood urea rises, and the excretion of nitrogen in the urine is decreased. Studies concerned with the excretion of nitrogen, which were made before the cortical hormone was available, were carried out with the animal in a state of acute suprarenal insufficiency. It is only when the animal is maintained with adequate amounts of cortical hormone, and after the basal metabolic rate is increased by the administration of thyroxin, that the influence of the cortical hormone on nitrogen metabolism can be demonstrated.

Explanation of effect of thyroxin on a normal animal. Large amounts of cortical hormone have been shown in the present experiments to prevent or to lessen greatly the negative nitrogen balance which ordinarily develops when two 10 mgm. doses of thyroxin are administered intravenously on consecutive days. A new factor, therefore, becomes apparent, which

may exert an influence on the amount of nitrogen lost following administration of thyroxin to a normal animal, namely, the amount of cortical hormone available. Sometimes, as was demonstrated in the case of the female dog (fig. 5), a negative nitrogen balance is not produced in a normal dog by such doses of thyroxin as were employed in these experiments. This suggests that when thyroxin is administered to a normal animal, as described, either the suprarenal cortex is stimulated, so that increased quantities of cortical hormone are produced, or else the dog normally is producing a sufficiently large amount of cortical hormone to reduce to a minimum, or to prevent, the negative nitrogen balance.

CONCLUSIONS

In these experiments, the only factor modifying the effect of thyroxin on nitrogen metabolism apparently is the quantity of cortical hormone administered. Therefore, the conclusions which may be drawn as a result of this investigation are:

1. Complete excision of all cortical tissue does not necessarily produce a negative nitrogen balance.
2. Negative nitrogen balance may occur in a suprarenalectomized dog receiving a low maintenance dose of cortical hormone.
3. Administration of thyroxin to a suprarenalectomized dog receiving a low maintenance dose of cortical hormone produces a negative nitrogen balance.
4. The hormone of the suprarenal cortex exerts a sparing action against the effect of thyroxin on nitrogen metabolism. The negative nitrogen balance can be lessened and a positive balance sometimes can be maintained by administering, with the thyroxin, sufficiently large amounts of cortical hormone.
5. The hormone of the suprarenal cortex lessens the severity and duration of the systemic reaction following the administration of thyroxin.
6. Experimentally, it has been demonstrated that the amount of cortical hormone administered is one of the factors which determines the amount of loss of nitrogen produced by a given dose of thyroxin. The results suggest that the effect of thyroxin on nitrogen metabolism may be indicative of the amount of cortical hormone available in the body of the dog.
7. Enlargement of the thymus is uniformly observed in dogs which have survived bilateral suprarenalectomy for two months or longer. The relation of thymic hyperplasia to nitrogen metabolism has been neither proved nor disproved.

PROTOCOLS. *Experiment 1.* An adult female hound weighed 17.1 kgm. at the time of operation. While the dog was under ether anesthesia, the right suprarenal gland was removed easily with its capsule intact, March 22, 1933. Studies of nitrogen

balance were started June 1 and were continued until September 27. The results obtained after three series of injections of thyroxin during the significant phase of this experiment are given in figure 1. Left suprarenalectomy was performed while the dog was under ether anesthesia September 27. This gland broke during removal, but all suprarenal tissue was removed. At this time the dog weighed 20.2 kgm. Daily injections of cortical hormone were given from the day of the second operation. Studies of nitrogen balance were continued until December 27. The animal subsequently died while receiving daily injections of thyroxin and a small dose of crystalline suprarenal cortical hormone. At necropsy, the animal was found to be well nourished. The peritoneal and pleural cavities appeared to be normal. The thymus was definitely enlarged and weighed 38 grams. All of the abdominal organs appeared to be normal and no change characteristic of acute suprarenal deficiency could be found. No accessory suprarenal cortical tissue was present. There was a small abscess on the thoracic wall, at the site of previous injections of the suprarenal cortical hormone. The exact cause of death could not be determined.

Experiment 2. Right suprarenalectomy was performed on an adult male mongrel October 13, 1932, while the animal was under ether anesthesia. Left suprarenalectomy was performed under ether anesthesia, January 31, 1933. The animal weighed 17.4 kgm. Daily injections of suprarenal cortical hormone were made, starting on the day of the second operation and continuing until the animal died. Studies of nitrogen balance were started in February and were continued until July 6. Results obtained after two series of injections of thyroxin in the significant phase of this experiment are given in figure 2. The animal died July 6, the cause of death being acute suprarenal deficiency. At necropsy, the animal appeared to have been well nourished; it weighed 22.6 kgm. Accessory suprarenal tissue was not present. The animal had been dead approximately twenty-four hours at the time of necropsy and postmortem changes already had occurred. The left pleural cavity contained about 300 cc. of yellowish-brown fluid. Because of autolysis of tissues, gross changes in the liver or pancreas, which are suggestive of suprarenal deficiency, could not be detected.

Experiment 3. Right suprarenalectomy was performed on an adult female shepherd dog, while the animal was under ether anesthesia, March 14, 1933. The gland was removed with its capsule intact. The animal weighed 12.3 kgm. Studies of nitrogen balance were started July 11, when the animal weighed 14.7 kgm. Results of studies from July 11 to September 26, during which time four series of injections of thyroxin were administered, are given in figure 3. On September 27 left suprarenalectomy was performed while the animal was under ether anesthesia. This gland likewise was removed with its capsule intact. The animal weighed 16.5 kgm. Daily injections of suprarenal cortical hormone were given, starting on the day of the second operation and continuing until the animal died. During the three and a half months following the second operation, studies of nitrogen balance were continued. Three series of injections with thyroxin were given in this period. Results of the studies are illustrated in figure 4. The animal died on May 5, 1934, during the course of some other experiments. At necropsy, the cause of death was found to be acute suprarenal deficiency. The pancreas was typically congested, and in the mucosae of the stomach and colon, a few hemorrhagic erosions were found. The liver did not appear to be as congested as is usually the case in animals dying of suprarenal deficiency. The thymus was much enlarged and weighed 32 grams.

Experiment 4. An adult female shepherd dog weighed 9.3 kgm. Studies of nitrogen balance were started April 27, 1933, and were continued until May 10. One series of injections of thyroxin was given in this period. Results of this study are

given in the left half of figure 5. The right suprarenal gland was removed May 11, while the animal was under ether anesthesia. At this time the animal weighed 9.5 kgm. Studies of nitrogen balance were started after the dog had recovered from operation and were continued until August 22. Results following administration of one series of injections of thyroxin are given in the right half of figure 5. On August 24, while the dog was under ether anesthesia, the left suprarenal gland was removed completely. The animal weighed 10.9 kgm. at this time. Daily injections of suprarenal cortical hormone were given, starting on the day of the second operation and continuing until the animal died. After the animal had recovered completely, studies of nitrogen balance were resumed and were continued until December 9, on which date the animal died. Results following three series of injections with thyroxin are given in figure 6. The cause of death was abscess beneath the liver, and generalized peritonitis. At necropsy, the animal weighed 11.7 kgm. The thymus was enlarged and weighed 45 grams. The heart, lungs, and thyroid gland appeared to be normal grossly. Accessory suprarenal tissue was not found. The pancreas and liver also appeared to be normal grossly. In the stomach and duodenum was a large amount of sanguineous fluid. Beading of the costochondral junctions, hemorrhage into muscles or joints, loosening of the teeth, or other evidences of scurvy, were not observed. Microscopic sections of the kidneys, thyroid gland, liver, and pancreas appeared to be normal.

Experiment 5. The right suprarenal gland was removed from an adult male police dog September 2, 1931 and the left suprarenal gland was removed July 18, 1932, while the animal was under ether anesthesia in each instance. At the time of the second operation, the animal weighed 22.4 kgm. The animal received daily injections of suprarenal cortical hormone. Studies of nitrogen balance were started April 10, 1933 and were continued until the animal died on November 29, 1933. From May until November 25, two 10 mgm. doses of thyroxin were administered intravenously on consecutive days, five different times. Sufficient cortical hormone had been administered with each test so that no untoward symptoms were produced. As a matter of fact, a marked negative nitrogen balance had not been produced. Therefore, the administration of the cortical hormone was discontinued for four days, beginning November 22. On November 26, 10 mgm. of thyroxin were injected intravenously and the same dose was repeated the following day. On November 27, the animal vomited slightly. On November 28, in the morning, the animal appeared to be suffering from suprarenal deficiency and 5 cc. of a potent solution of suprarenal cortical hormone were injected. The animal was seen again at 11 p.m. and appeared improved; there seemed to be no doubt that it would live until morning. At 8:00 a.m. the following day, the animal was found warm, but dead. The cause of death was acute suprarenal deficiency produced by the administration of thyroxin. At necropsy, the animal appeared to have been in excellent physical condition; its body was moderately obese, very muscular, and weighed 35.6 kgm. The thymus was greatly enlarged, weighed 80 grams, and appeared to be somewhat nodular. The heart and lungs appeared normal grossly. The thyroid gland was smaller than would have been expected and grossly appeared to have undergone slight degeneration. Hemorrhagic lesions were not present in the stomach, duodenum, or colon. The liver which weighed 600 grams, appeared to be normal. The pancreas was slightly congested. The kidneys were normal grossly. Accessory cortical tissue was not found. Loosening of the teeth, beading of the costochondral junctions, hemorrhages into muscles or joints, or other evidences of scurvy, were not present. Microscopically, the thyroid gland appeared to be normal. Sections of the liver-pancreas, kidneys, and thymus appeared to be normal.

Experiment 6. The right suprarenal gland was removed from an adult female mongrel January 13, 1933, and the left gland was removed March 7, 1933, while the animal was under ether anesthesia in each instance. Studies of nitrogen balance were made from March 8 to May 11. On March 31 the animal received an intravenous injection of 5 mgm. of thyroxin, and the same dose was repeated on two subsequent days. Starting April 16, the same daily doses of thyroxin were repeated, except that four injections were administered instead of three. During both series of injections, adequate amounts of suprarenal cortical hormone were administered and the animal tolerated the experiments well. On April 21 the daily dose of cortical hormone was reduced to 2 cc.; this amount proved to be adequate for maintenance. On May 7, the animal received an intravenous injection of 10 mgm. of thyroxin; the dose of suprarenal cortical hormone at this time was 2 cc. This injection was repeated on the following day. The dog remained in good condition until May 10, when the level of urea rose to 125 mgm. per 100 cc. of blood. On the afternoon of this day, 10 cc. of cortical hormone were administered. Nevertheless, on the following morning the animal was found dead. The cause of death was acute suprarenal deficiency produced by the administration of thyroxin. At necropsy, the thymus was enlarged and markedly congested. The lungs and heart appeared to be normal. The mucosae of the stomach and duodenum were moderately congested, but ulcerations were not found. The mucosa of the colon was hemorrhagic, and a considerable quantity of bloody fluid was present in the intestine. The liver was slightly congested. The pancreas was markedly congested. The kidneys appeared to be normal. The mucosa of the urinary bladder was hemorrhagic. Accessory suprarenal tissue was not found.

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ON THE MECHANISM OF PHOTOSENSITIZATION IN MAN

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Considerable confusion has existed in the past regarding the relationship of the phenomena resulting from the photosensitization of living systems by dyes to normal and abnormal photo-physiological effects in man. Such photosensitization has been generally termed *photodynamic action* and this term will be used for convenience to designate this type of reaction exclusively. Blum and Spealman have recently (1934) reviewed the evidence which has accumulated to show that photosensitization by dyes is dependent upon the presence of molecular oxygen, a differential point between such processes and the effects of ultra-violet light which are independent of O_2. At that time, no experiments had been performed to demonstrate the requirement of O_2 for photodynamic effects in man. We have devised a simple method for the demonstration of this fact in locally sensitized areas of human skin, and have used it to show the difference between such artificial sensitization and that occurring in a case of pathological photosensitivity.

Experimental. In these studies we produced local photosensitization of the skin by intradermal injection of photoactive dyes, following the method of Duke (1923) and Frei (1926). We employed both rose bengale and hematoporphyrin as sensitizers; rose bengale[1] was used in 10^{-4} M concentration in phosphate buffer pH 6.7; hematoporphyrin solution was made up from the commericial preparation *Photodyn*[2] (Nordmark) diluted 1:20 with phosphate buffer. The quantity of solution injected was 0.1 cc. After an hour any reaction resulting from such injections has usually subsided, leaving an area 4 or 5 mm. in diameter faintly colored by the dye. If not exposed to light this dye disappears completely after a few days leaving little or no trace.

When exposed to bright sunlight a distinct wheal forms over the area injected with dye, surrounded by a red flare having the characteristics of an urticarial reaction or the "triple response" of Lewis (1927). In most cases the formation of the wheal is accompanied by severe itching.

[1] Tetra-brom-tetra-chlor fluorescein.

[2] Kindly donated by Dr. Harold D. Palmer.

Usually 3 to 5 minutes of exposure serve to elicit the response, which may not appear, however, for several minutes following the irradiation. The intensity of whealing varies greatly with the period of exposure and with the individual.

Effect of O_2 lack. To test the effect of low O_2 tension we made one or more injections into each forearm and then, a few hours later, occluded the circulation to one arm by means of pressure in a sphygmomanometer cuff. After the circulation had been cut off for 5 to 8 minutes both forearms were exposed to sunlight for 4 to 5 minutes. The pressure in the cuff was then released. The triple response never appeared on the arm which had been deprived of its circulation in this way. The response ap-

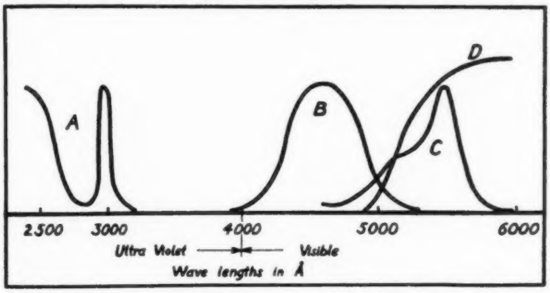

Fig. 1. Spectral regions producing three types of photo-response in human skin.

A. Erythema and pigmentation of normal skin.

B. "Triple response" of *urticaria solare* (curve roughly approximate).

C. Absorption spectrum of rose bengal, hence, the spectral region to which the skin is sensitized by injection of this dye. There is a further absorption in the ultraviolet which is not shown.

D. Transmission of Corning filter 338. Wave lengths to the left of D are not transmitted, hence response B is virtually eliminated by this filter while response C is not.

Abscissa: wave lengths in Å.

Ordinates: arbitrary values.

pearing on the arm with circulation intact served as a control. This experiment gives a simple and definite proof of the requirement of O_2 for *photodynamic* effects in the skin of man.

Relation of urticaria solare. Blum, Allington, and West (1935) have described the case of an individual who reacts to blue light (4000–5000 Å) with a very pronounced "triple response" (urticaria solare). Suspecting that this might be a sensitization of the *photodynamic* type, they attempted to prevent the response by occluding the circulation by the method used above. Although the circulation was cut off for as long as 15 minutes before the exposure to light, the response, which is evoked by exposures as short as one minute, was not diminished. At the time some doubt was

felt that sufficient anoxemia had been produced to inhibit a *photodynamic* effect. To answer this question we produced artificial sensitization in this individual by injecting rose bengale locally, following the above procedure. We then exposed these areas to sunlight through a glass filter (Corning 338) which cuts out all wave lenghts shorter than 4900 Å, i.e., virtually all the radiations which evoke the abnormal triple response in this individual, but allow the major part of the visible radiation absorbed by rose bengale (maximum 5500 Å) to pass. Figure 1 shows the spectral relationships involved. We were able in this way to produce the typical wheal at the point of injection of the rose bengale on the arm with circulation intact, but not on the arm with circulation occluded for periods as short as 5 minutes. There can thus be little doubt that the occlusion of the circulation by means of the sphygmomanometer cuff may produce sufficient anoxemia to prevent sensitization of the *photodynamic* type in this individual, whereas the abnormal response cannot be prevented in this way. Hence we must conclude that *urticaria solare* is not a sensitization of the *photodynamic* type.

Relation to the normal ultra-violet erythema. We have tested the effect of O_2 lack on the normal erythemic response elicited by ultra-violet radiation shorter than 3200 Å. When the circulation to one arm of an individual was occluded, and both arms were subjected to a strong erythema dose of quartz-mercury arc radiation, erythema appeared in approximately equal intensities on both arms. The circulation was occluded for 5 minutes before, and maintained throughout the irradiation. It is impossible to state that the O_2 was completely removed from the region of the skin to which these radiations penetrate, but all other evidence leads us to believe that the effect of such radiations upon living systems is independent of the presence of O_2 (see Blum and Spealman, 1934). It seems therefore that the normal erythemic response of human skin should be considered as having no relationship with *photodynamic action.*

Pigmentation. Numerous investigators have reported heavy pigmentation of the skin of men injected with acridine dyes who were subsequently exposed to sunlight (see Hausmann and Haxthausen, 1929), and it has been often assumed that a relationship exists between the mechanism of this pigmentation and that normally following exposure to ultra-violet radiation of the normal erythema producing wave lengths. It is therefore interesting to remark that the pigmentation of local photosensitized areas follows the wheal formation resulting from exposure of these areas to light. Wheal formation is not ordinarily followed by pigmentation as is common experience with urticarial responses in general, and the abnormal triple response of *urticaria solare,* although very severe, is never followed by the slightest trace of pigmentation. This is not due to a lack

in pigment mechanism of the individual with *urticaria solare*, who pigments normally when exposed to the quartz-mercury arc.

Whatever the source of the *urticaria solare* response, it seems probable that the *photodynamic* "triple response" probably results from the local appearance of histamine or "H" substance due to tissue injury (see Lewis, 1927). It is probable that the pigmentation following this response is likewise a result of tissue injury, and is thus related to that following burns or other skin lesions. Its relationship to the normal pigmentation of skin following ultra-violet irradiation would appear to be obscure, since there can be little connection between the photo-chemical processes which produce the two phenomena.

Discussion. It would seem well to summarize the differences in the three types of photo-response described above:

A. *Normal erythema* is produced by ultra-violet radiation of wave lengths less than 3200 Å. This is a delayed reaction, erythema appearing an hour or more after moderate exposures; it is followed later by pigmentation. Both erythema and pigmentation are independent of O_2.

B. *Urticaria solare* manifests itself as a "triple response." It is produced by visible light (4000–5000 Å). The response is immediate, whealing occurring in a very few minutes. It is not followed by pigmentation, and is independent of O_2.

C. The *photodynamic* "triple response" follows artificial photosensitization, and is similar in appearance to B. It is produced by those wave lengths absorbed by the particular sensitizer. The response is immediate, whealing occurring within a few minutes. It is followed by pigmentation and does not occur in the absence of O_2.

Figure 1 illustrates graphically the spectral relationship of these three responses. It is interesting to note that it was possible to produce all three in a single individual.

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EXCRETION OF INULIN, CREATININE, XYLOSE AND UREA IN THE NORMAL RABBIT

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The present paper is a brief report of renal clearances in the rabbit, the intent of these observations being to determine to what extent this animal, in respect to renal activity, parallels the more extensively studied dog and man.

METHODS. Male rabbits weighing from 2 to 4 kgm., obtained directly from the farms and kept in the laboratory on a constant diet for about one week, were used for these observations. The diet consisted either of cabbage and carrots, or 4 to 5 ounces of Ralston Purina rabbit chow, with water *ad libitum*.

All food, but not water, was kept from the rabbits 18 hours before each experiment. On the morning of the experiment the animal was given additional water by stomach in varying quantities, usually 40 cc. per kilogram repeated three times at half-hourly intervals.

In those instances where the excretion of xylose was followed, this sugar was given in doses of 2 grams per kilogram administered by subcutaneous injection of a 4 per cent solution, 70 to 90 minutes before the start of the urine collection period. We used subcutaneous injection since we were unable to achieve adequate plasma xylose levels with 2 grams per kilogram administered by stomach. The plasma xylose concentrations after subcutaneous injection reached 125 to 150 mgm. per cent at the start of the experiment and fell slowly to 70 mgm. per cent at the end of 3 hours.

Inulin was administered in two doses. One gram per kilogram was given by subcutaneous injection 100 to 110 minutes before the first urine collection period; a second dose of 2 grams per kilogram was given intravenously in the marginal ear vein 45 minutes before the first period; for both injections we used a 20 per cent solution in 0.6 per cent NaCl, prepared according to Shannon and Smith (1935). Creatinine was administered subcutaneously as a 5 per cent solution 50 to 60 minutes before the first urine collection period in doses ranging from 50 mgm. to one gram per kilogram, depending upon the desired plasma creatinine value.

All urines were collected by catheterization, using an infant male catheter F12. The bladder was emptied at the start of the first period and

urine collections made at 10 to 30 minute periods, depending upon the rate of urine formation. The bladder was not washed out, for it appeared in sacrifice experiments that we could obtain complete emptying by first draining through the catheter, and then inflating the bladder two or three times with air and expressing the latter, with the catheter in place, by firm pressure over the suprapubic area. At very low urine flows the urine was expressed from the bladder while a graduated centrifuge tube was held below the penis; air was then introduced by catheter, and the bladder emptied again after the catheter had been removed. The urine was immediately diluted to the expected U/P ratio and precipitated as described under chemical methods. Blood samples were collected in most instances at 50 to 60 minute intervals by puncture of the femoral or the marginal ear vein, from 1 to 5 cc. of blood being taken, according to the amount required for analysis. The blood was treated with heparin and centrifuged immediately. In those experiments where inulin was administered intravenously, all blood samples were taken by heart puncture at approximately the midpoint of each urine collection period. Plasma curves were constructed for all substances examined and plasma values were obtained by interpolation to the midpoint of each urine collection period.

All chemical procedures were the same as those used by Shannon and Smith (1935) with the exception of the analyses for sugars where the determinations were done by the Folin (1929) method.

Throughout the work reported in this paper, calculations are given per square meter of body surface (S.A.). To arrive at a suitable constant for the calculation of this term we used the figures of Taylor, Drury and Addis (1923); by plotting their data for observed surface area against body weight, we obtained a constant of 8.9 as an average for rabbits between 2 to 4 kilograms.

We have found it difficult to maintain a high rate of urine flow in the rabbit. If water in quantities greater than three successive doses of 40 cc. per kilogram at 30 minute intervals is given by stomach, the animal is apt to develop a paradoxical oliguria, which is not infrequently followed by convulsions, characterized by opisthotonos, and death. The phenomena early suggested that we were dealing with the water intoxication described by Rowntree (1923). We are inclined to believe that the intravenous injection of various substances (creatinine, inulin, etc.) favors this reaction, which perhaps reflects an inability to prevent excessive dilution of plasma constituents by adequate diuresis (see Smirk, 1932). Helwig, Schutz and Curry (1935) have recently described convulsions and death in rabbits in consequence of the administration of water by rectum.

Discussion. In table 1 there is given a series of 42 observations on the simultaneously determined clearances of creatinine and inulin in the

TABLE 1

Simultaneous creatinine and inulin clearances in the normal rabbit

NUMBER OF RABBIT	WEIGHT	S. A.	URINE FLOW	PLASMA		CLEARANCE		CLEARANCE RATIOS
				Creatinine	Inulin	Creatinine	Inulin	Creatinine / Inulin
	kgm.	*sq. m.*	*cc./sq. m./min.*	*mgm. per cent*	*mgm. per cent*	*cc./sq. m./min.*	*cc./sq. m./min.*	
2	3.00	0.182	0.62	9.4	270	54.0	54.9	0.98
			0.17	9.1	250	14.3	15.4	0.93
			0.21	7.1	211	24.5	24.2	1.01
			0.09	6.4	193	9.2	9.2	1.00
8	3.50	0.205	2.20	9.8	214	75.5	77.1	0.98
			4.10	8.9	162	56.1	56.3	1.00
			5.60	9.0	135	59.4	61.0	0.97
4	2.80	0.177	0.96	9.7	248	48.0	54.1	0.89
			0.43	9.5	212	35.0	35.3	0.99
			0.19	8.6	178	21.6	21.9	0.99
10	3.25	0.187	0.87	10.1	179	40.7	41.8	0.97
			0.87	10.8	177	32.3	35.9	0.90
			0.50	10.7	155	23.5	24.0	0.98
11	3.75	0.215	0.87	12.4	299	19.6	20.3	0.97
			0.81	11.9	292	19.1	18.7	1.02
9	3.55	0.207	0.43	16.5	350	24.3	22.9	1.06
			0.18	15.2	260	9.3	9.3	1.00
4	2.90	0.181	0.61	16.5	454	48.5	47.0	1.03
			0.33	15.1	367	25.9	26.1	0.99
			0.41	11.4	173	40.5	41.4	0.98
3	3.00	0.182	3.08	28.3	275	53.3	55.2	0.97
			1.76	24.6	218	44.5	42.3	1.05
			0.82	20.5	169	41.8	38.5	1.08
			0.77	19.2	141	39.8	36.3	1.10
1	2.75	0.174	3.56	31.9	188	38.3	43.0	0.89
			5.00	30.0	136	46.1	48.6	0.95
			3.45	28.0	101	39.6	42.2	0.94
			2.30	26.2	83	36.7	37.6	0.98
			2.18	40.0	334	31.9	32.2	0.99
			2.47	41.0	279	33.3	33.6	0.99
4	2.30	0.155	3.42	48.7	200	56.5	60.0	0.94
			2.71	51.2	188	53.9	55.7	0.97
			0.97	45.7	159	43.7	43.2	1.01
			0.65	43.0	138	28.2	27.9	1.01
7	3.35	0.199	1.15	82.6	199	52.1	54.3	0.96
			0.78	71.5	162	46.2	47.2	0.98
5	2.50	0.164	2.07	89.4	276	55.7	58.0	0.96
			1.52	85.1	218	47.8	45.7	1.04
6	2.50	0.164	0.82	109.5	405	24.6	25.6	0.96
			0.33	124.5	465	6.5	6.5	1.00
			0.30	122.0	430	5.9	5.9	1.00
			0.40	125.5	435	6.3	5.9	1.07

Average, 0.99;
Standard deviation, 0.0415.

normal rabbit. The creatinine/inulin clearance ratio averages 0.99, with a standard deviation of 0.0415. According to the data in table 1, the equality between the creatinine and inulin clearances is maintained at all levels of plasma creatinine ranging from 6 to 125 mgm. per cent, and at urine flows ranging from 0.1 cc. to 5.0 cc. per square meter per minute (or from inulin U/P ratio of 10 up to 105).

In view of the evidence presented in previous publications from this laboratory, especially in the paper by Shannon and Smith (1935), the

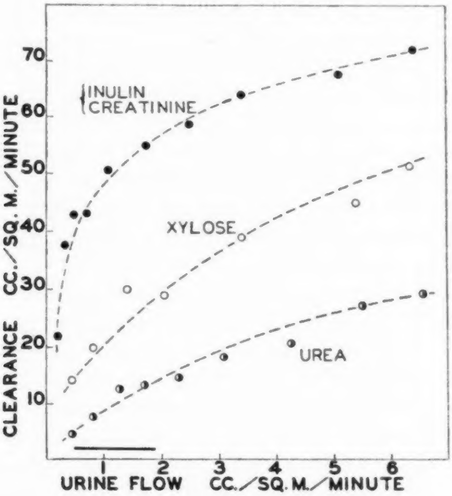

Fig. 1. Inulin, creatinine, urea and xylose clearances in the rabbit, expressed in relation to urine flow. Each datum represents the average of 10 observations selected within a particular range of urine flow. These clearances were not all simultaneously determined and represent a number of different animals. The horizontal bar indicates the approximate range of normal urine flow, as based upon 24 hour collection with water *ad libitum*. The dotted lines are drawn simply to emphasize the trend of the data.

inulin clearance is accepted here as equal to the rate of glomerular filtration. We have not examined the influence of plasma level of either inulin or creatinine on the respective clearances, but MacKay and Cockrill (1930) have established this relationship in the rabbit for creatinine (see below). In view of the fact that no curvilinear relationship exists between the creatinine/inulin ratio and the plasma creatinine concentration, our observations, according to the arguments advanced for the dog by Shannon (1935), confirm MacKay and Cockrill's conclusion in indicating no tubular secretion of creatinine in this animal.

In addition to the data presented in table 1, we have series of 70 simultaneous clearances of urea and xylose; 60 independent creatinine clearances; 20 independent urea clearances, and 6 simultaneous xylose-inulin clearances. There is no doubt that different animals show differences in renal activity, even when compared on the basis of surface area, but in view of the fact that at the same urine flow the clearances of any one substance have about the same magnitude in different animals, we feel justified in considering as a uniform series all clearances of any one substance.

In figure 1 we have summarized 260 observations on inulin, creatinine, xylose and urea, in relation to the rate of urine formation. Each datum in the figure represents the average of ten observations selected within a given range of urine flow. It is evident from these data that the clearances of all substances are markedly affected by alterations in urine flow. There is no evidence of a tendency for the clearance of any substance to become constant at higher rates of urine formation, unless it is above 6 cc. per square meter per minute, and the latter possibility is unlikely since this figure is nearly the maximum rate of urine formation in unanesthetized rabbits after water *per os*.

It is particularly interesting that the clearances of creatinine and inulin vary with urine flow; in fact, these clearances fall precipitously at urine flows below 1 cc. per square meter per minute. It is difficult to believe that this variation is attributable, in any significant degree, to either passive or active reabsorption of these substances; the large size of the inulin molecule, and the identity of the creatinine and inulin clearances under all conditions, as pointed out above, would indicate that the decreased rate of excretion of these substances at low urine flows is due to a decrease in the rate of glomerular filtration. That a change in renal blood flow in the rabbit may accompany changes in rate of urine formation is suggested by the observations of Mayrs and Watt (1922). These investigators measured renal blood flow in the rabbit by a direct method, and when one plots their values for blood flow against the observed urine flows, one finds suggestive correlation between these terms. The observation of Richards and Plant (1922) on the perfused kidney, and of Khanolkar (1922) and Hayman and Starr (1925) on kidneys injected with hemoglobin or Janus green, indicate that diuresis in the rabbit is accompanied by an increase in glomerular circulation. Though none of these lines of evidence is indubitable, it seems to us that the present data on the excretion of inulin and creatinine point very strongly to a physiological association between glomerular function and urine flow in the rabbit.

Accepting the above interpretation, it is the change in glomerular filtration that accounts in great part for the decrease in the xylose and urea clearances at low urine flows. But that there is, in addition, extensive

reabsorption of these substances is indicated by the facts that the deficit between these clearances and the creatinine-inulin clearances becomes greater at lesser urine flows, and that even at maximal urine flows, this deficit is still marked. In our simultaneous observations, the urea/xylose clearance ratios vary from 0.60 at 6 cc., to 0.32 at 0.5 cc. per square meter per minute, while the simultaneous xylose/inulin clearance ratios vary from 0.68 at 6.0 cc. to 0.56 at 3.0 cc. per square meter per minute. Since we were unable to maintain the rate of urine formation after the administration of phlorizin, it has not been possible to determine to what extent the deficit in the xylose clearance is due to active reabsorption, but it is clear that a somewhat larger proportion of the pentose is reabsorbed from the glomerular filtrate in the rabbit than in the dog or man. A still greater proportion of urea is reabsorbed, and in both instances the reabsorbed fraction increases as the rate of urine formation decreases.

It is clear that the rate of urine formation is related to general renal activity in the rabbit in a manner quite different from the relationship that has been described for man or dog. It is quite impossible to designate any point as an "augmentation limit" (such as has been described for urea in man by Austin, Stillman and Van Slyke (1921), and in the dog by several investigators). It appears that in the rabbit changes in urine flow are mediated in large part by changes in glomerular activity, in addition to changes in the tubular reabsorption of water. Since the administration of water either by mouth or by injection can lead to oliguria, it is difficult to make systematic observations at high urine flows by this method.

We have made no effort to relate the absolute clearances of any of these substances to their respective plasma levels. The excretion of any substance which measures glomerular filtration will be linearly related to its plasma level only if glomerular filtration is maintained at a constant value. That this constancy can be maintained under experimental conditions is indicated by the findings of a linear relationship between plasma level and rate of excretion in the rabbit for creatinine by MacKay and Cockrill (1930), for urea by Addis, Barnett and Shevky (1918) and for creatinine and sucrose by Cope (1933). It should be noted that these results were obtained with a carefully standardized technique, such as was early used by Addis and his co-workers to obtain "full or maximal" renal activity in the rabbit. On the other hand, Kay and Sheehan (1933) have found that the renal extraction ratios of urea and creatinine were related to the plasma levels of these substances. The evidence presented in this paper concerning the identity of creatinine and inulin clearances makes the observations pertaining to creatinine, at least, best explicable by glomerular factors, rather than by changes in either tubular secretion or reabsorption.

SUMMARY

Observations on the inulin, creatinine, xylose and urea clearances in the normal rabbit are reported. All of these clearances increase with increasing urine flow, and fail to reach a constant value at urine flows that are maximal under the conditions of these experiments.

Simultaneous clearances of inulin and creatinine are equal, regardless of urine flow or plasma level on creatinine. In view of this and previous evidence on the excretion of these substances, the change in the inulin and creatinine clearances in relation to changing urine flow is interpreted as indicating a corresponding change in glomerular activity.

The xylose and urea clearances, both in simultaneous and independent observations, are considerably less than the creatinine-inulin clearance.

When water is administered by stomach in excessive amounts it leads paradoxically to oliguria, convulsions and death. Water intoxication appears to be favored by intravenous injections. This phenomenon, coupled with the physiological relationship between glomerular activity and water excretion, should be considered in all renal function studies in this animal.

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THE NERVE CONTROL OF THE CORONARY VESSELS WITH NEW EXPERIMENTAL EVIDENCE FOR THE PATHWAYS OF EFFERENT CONSTRICTOR AND DILATOR NEURONES IN THE DOG[1,2]

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The mechanisms coördinating the degree of cardiac activity with the volume of the coronary circulation have not yet been adequately demonstrated.[3] However, the presence of efferent coronary nerves has been described by a number of authors, and some evidence of coronary reflexes has been published. The present report undertakes to verify and extend experimental evidence of the efferent paths of coronary nerves. Data for the mechanisms of reflex coördination in the control of the coronary circulation will be offered in a later paper. The two papers present experimental results indicating a most delicate control of the coronary circulation, a regulation which must form the basis of an adequate respiratory and nutritional metabolism of the heart as a muscular organ.

Martin and Sedgwick in 1881 (2) demonstrated the correlation of pulse variations and blood pressures in the coronaries and the systemic arteries. They were first to observe the effects of coronary vasomotor nerves. By direct visual inspection they noted changes in the size of coronaries from which they inferred nerve influence. However, their experiments, staged in the search for proofs, were "entirely negative." Ten years later Martin (3) announced that the coronary arterioles could be observed under a lens to dilate upon stimulating the vagus nerve, and also upon asphyxiation.

[1] Experiments on the innervation and chemical control of the volume of the coronary blood flow have been performed in several series of dogs in studies extending through five years. Preliminary reports have been made from time to time, but it is planned now to present the more comprehensive results in a series of articles.

[2] We acknowledge liberal grants in partial support of the expenses of this investigation from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, of the American Medical Association.

[3] The increase in work of the body expressed in the oxygen consumed, the coincident rise of blood pressure and heart rate, and the augmented minute-volume of blood moved by the heart, have been shown to be delicately synchronized, Cotton, Rapport and Lewis (1). The associated and equally important responses of adjustment in coronary volume to variations in cardiac work are yet to be determined quantitatively. Qualitative data follow in the present report.

Porter, 1896 (4) must receive credit for the first experimental demonstration of coronary vaso-constrictor nerves. On stimulating the cervical vagus Porter observed a decrease of overflow from an open coronary from 13 drops to 8 drops per 15 seconds, with return in the after-period to the preceding rate of flow. Porter's demonstration has become classic, first, for showing an active vasomotor regulation where only passive fluctuations in coronary flow had previously been observed; and second, for demonstrating the presence of vaso-constrictor nerves in a parasympathetic nerve trunk.

Maass in 1899 (5) offered detailed experimental evidence of the presence of dilator nerves to the coronary arteries in cats. The coronary dilator nerve pathway was via the thoracic sympathetics, the stellate ganglion, the ansa and the associated cardiac nerve branches. He measured the total venous outflow from a cannula in the right descending vena cava of the isolated heart. In certain tests Maass obtained coronary reactions of opposite sign both from the vagus and from the thoracic sympathetics, which left him in doubt as to the true paths of outflow of coronary nerves.[4]

Wiggers (6) in 1909 observed coronary constriction and made the first graphic tracings.

Morawitz and Zahn (7), 1912, devised the method which we have adopted of inserting a cannula directly into the coronary sinus, occluding the sinus by an inflated balloon. This method yields a continuous record of the fluctuations in rate of discharge of the group of coronary vessels that empty into the sinus.[5] The method is adapted to studies of the heart in situ, without undue disturbance of the natural nutritional and respiratory relations of the entire nervous system of the intact animal, as well as of the heart. While the use of anesthesia is unavoidable, the maintenance of the general circulation and of nervous sensitivity near the normal reflex level makes possible the determination of reflex coronary control.

Morawitz and Zahn established in dogs and cats that the coronary flow varies passively with arterial pressure, in whatever way the pressure change be induced. Also that the coronary sinus delivers only about 60 per cent of the total coronary blood, as shown also by Markwalder and Starling (8). By stimulating the cardiac accelerans, they induced active dilatation of the coronaries to an extent that left no doubt of the presence

[4] "In manchen Fällen lässt sich durch Vagusreizung der Blutstrom beschleunigen; es muss also angenommen werden, dass in ihm ausser gefässverengernden Fasern auch gefässerweiternde für das coronargebeit verlaufen," Maass (5, p. 294).

[5] Morawitz and Zahn (7) reported their coronary discoveries at the Congress für innere Medizin at Wiesbaden in 1913, describing their new cannula. Professor Starling applied the method in a study of the proportion of the coronary blood returning via the coronary sinus and announced the results in 1913 under the authorship of Markwalder and Starling (8).

of specific dilator fibers. Stimulation of the cervical vagus in their tests usually reduced the coronary flow. Yet they believed that proof of vagal content of coronary constrictor fibers is "uncertain and awaits future research."

Anrep (9) (10) and colleagues applied the anemometric method to the problem of coronary physiology. The method records with delicate sensitivity cardiac cyclic events of brief duration. The method was used to confirm and elucidate the following: Coronary constrictor reactions upon vagal stimulation, coronary dilatation on stimulating the stellate pathway, intimate but passive dependence of coronary flow on aortic pressure, cyclic variations in coronary flow in response to external pressures from the contracting myocardium on the coronary vessels, heart rate, systemic minute-volume and coronary flow are independent functions; and that coronary flow increases upon stimulating the central end of the vagus, due to reflex reaction.

Experimental methods. The present report is based on observations in more than 4,000 tests. We have published a number of preliminary reports on different phases of the work as the problems have developed (11–15).

The active coronary reactions recorded in the paper were determined by the method of Morawitz and Zahn (7), modified in certain important respects. The cannula was bent to more exactly fit the curve of the sinus (fig. 1) and its tip was perforated by a ring of openings which maintains a perfect drainage when the cannula is somewhat disaligned by outside mechanical influences.

Markwalder and Starling (8) computed the rate of flow from the coronary sinus as 60 per cent of the total coronary blood returning to the right auricle. The ratio varies in different animals but for our purposes the exact distribution of the returning coronary blood among the coronary veins in a particular animal has no significance to the problem of coronary control, if the variations are quantitatively in the same direction within the different coronary areas.

The computed rate from the recorded data has been expressed as cubic centimeters per minute. An increase of flow from the coronary sinus under guarded experimental conditions has been attributed to active coronary dilatation and vice versa.

All experiments to determine the active changes in rate of flow through coronary vessels must take into account the immediate mechanical influence of variations in aortic pressure. The coronary vessels are elastic tubes and the rate of coronary flow passively follows the slightest change in general arterial pressure. Variations in aortic pressure are absent or easily controlled in the isolated heart, and in the heart-lung preparation, but in the heart in situ they introduce at times almost unsurmountable

difficulties in the analysis of the active factors in vaso-motor control. This analysis has been developed by Martin and Sedgwick (2), Porter (4), Anrep and Segall (10) and others.

The arterial pressure has been equalized by the following apparatus: A tubulated Wolff bottle was mounted horizontally at a height of one meter. One tube drained off any excess of fluid from above the mark. A second tube of large diameter (5 mm.) was connected to the femoral artery. Just outside the arterial cannula a 100 cc. flask with 2-way stopper was inserted and kept warm in a water bath. The device has the advantage of equalizing arterial pressure under both types of blood pressure variation, since it receives overflow blood during constriction and returns excess blood to the vessels during systemic dilatation. For the present research, it is superior in these respects to the Knowlton and Starling method (16). The control device reduced the arterial pressure to a remarkably uniform level, as certain figures demonstrate (fig. 5). In extreme changes of resistance there is some lag in the control. On the whole reflex reactions are very satisfactorily equilibrated by the device.

The studies have been made on dogs of 5 to 10 kilos body weight. The dogs were narcotized, or anesthetized with ether, or chloretone, or Grahant's mixture of chloroform, alcohol and water, or morphine and ether. The Morawitz-Zahn method for coronary measurements requires an open chest, tracheotomy and artificial respiration. Volatile anesthetics have been given via the air pathway of the machine for artificial respiration.

The blood was prevented from clotting by heparin, 5 mgm. per kilo weight of animal, two-thirds of the heparin was given at the beginning and the remainder after thirty minutes.

A coronary manometer was constructed especially adapted to coronary tests. It was made of a large glass tube ground to a uniform diameter and to a volume of 0.7 cc. per millimeter length, a size favorable for animals of near 10 kilos in weight. The writing float of the manometer recorded on smoked paper without magnification. The manometer was mounted low enough to guarantee a small negative pressure at the coronary cannula. The coronary blood entered the manometer below the float. Periodically it was drawn off from the recording manometer and returned at a temperature of 37°C. to the right jugular vein by means of a Woodyatt pump. An electrically heated operating table and a warming pad aided in maintaining the normal body temperature under the adverse conditions of the open chest methods. Nevertheless, satisfactory reflex reactions were maintained for tests lasting several hours.

Mechanical records of coronary flow, arterial blood pressure, and the usual time and signals were assembled on an electrically driven belt kymo-

graph. The motor of this apparatus is synchronized and the speeds are well adapted to diagrammatic recording.[6]

Anatomical course of the efferent coronary nerves. The coronary vessels are supplied by a full complement of vaso-motor nerves. The vaso-dilator supply is from the thoracic sympathetic division of the autonomic system. The pathway from the thoracic cord is by way of the upper thoracic chain ganglia and the complicated anatomical pathways of the ansa, the vagus, the inferior cervical ganglion and the cardiac nerves that compose the cardiac plexus. The coronary vaso-constrictor supply is present in the cervical vagus trunk and the fibers course out through its branches into the cardiac nerves and cardiac plexus in closest anatomical peripheral association with the coronary dilators.

A challenge to experimental physiologists has long been the demand for observational details and proofs that would determine the more exact relation of these antagonistic systems, and clarify and answer the questions raised by Porter, and Morawitz and Zahn as to the exact course of the constrictors and the dilators respectively. Porter was suspicious that coronary dilatations could occur upon stimulating the cervical vagus trunk, but could not secure proofs. And Morawitz and Zahn were unable to answer the question of the presence of coronary constrictors as components of the sympathetic.[7] Each

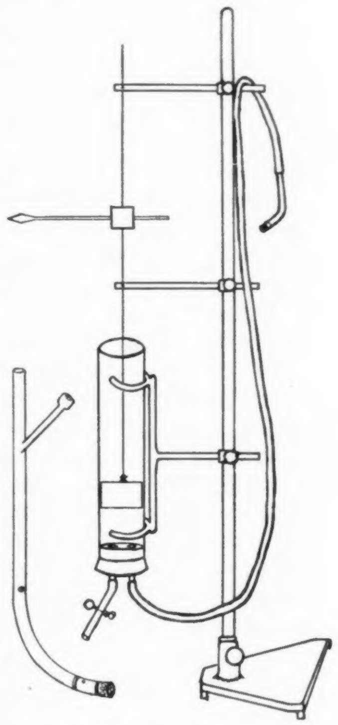

Fig. 1. Volume recorder devised for direct and continuous measurement of the blood flow from the coronary sinus. The manometer is used at a level below the sinus to insure a low pressure at the tip of the Morawitz cannula. The volume tube is ground to a uniform diameter of 30 mm.

The Morawitz cannula illustrated at the left shows the exact curve and the ring of openings at the tip which insures more perfect drainage of the sinus.

[6] An electrically driven recording kymograph of dependable uniformity of speed was constructed by the Harvard Apparatus Company to specifications growing out of this research. The author is under deepest obligation for this scientific coöperation.

[7] "Im Herzsympathicus müssen also Vasodilatatoren für die Kranzgefässe verlaufen. Ob daneben im Sympathicus auch vasokonstringierende Fasern für die Coronargefässe enthalten sind, lasst sich nicht sagen." Morawitz and Zahn (7, p. 398).

group of investigators was evidently confused by the presence in common trunks of both types of coronary neurones. The present report determines the more exact boundaries of each of the coronary efferent nerve groups, and examines the common nerve pathways wherever the two sets of axones overlap in the course of their distribution.

Reactions of the coronary vessels to peripheral nerve stimulation. In the dog the vagus nerve and the cervical sympathetic are in a common sheath. While the bundles of the respective components can be followed to some degree by histological methods, this cannot be accomplished physiologically except for a few millimeters of the vagus below the ganglion nodosum and the similar free portion of the cervical sympathetic immediately adjacent to the superior cervical ganglion. Even so there often is a direct connective between these two ganglia. This blending of bundles of neurones of very diverse type and origin from the central nervous system extends to all vagus and sympathetic branches both of the cervical and the upper thoracic areas that contribute to the cardiac plexus. It accounts for the experimental difficulties in determining the full facts of the course and character of nerves exercising coronary arterial control.

The customary technique of stimulating the cervical vagus or the thoracic divisions of the vagus yields the diverse reactions of the composite vago-sympathetic. In fact, in all research efforts to analyze the functions of individual components of the efferent cardiac arterial supply one must be prepared to identify and evaluate at least four principal functional nerve groups, namely, cardiac inhibitors of rate, cardiac accelerators, coronary arterial dilators, and coronary constrictors. The cardiac inhibitors can be eliminated at once by atropine, which does not influence the reactions of the other groups. We have freely used the atropine method in experimental tests.

The coronary reactive capacity varies with different animals. Young dogs are more responsive to efferent reactions, especially of the constrictor type.

Active coronary changes are obtained from either right or left vago-sympathetic systems. On the whole the left, see tables 1 and 2, produces a greater amount of constriction, a point not to be too strongly emphasized. The right seems to yield dilatations with greater ease and in greater volume.

Cohn (17) has shown an analogous variation in the two vagi in that the right vagus has a greater effect on stimulus production, while the left has a more profound influence on conduction.

Active coronary changes measured in terms of variation of rate of blood flow from the cannulized coronary sinus are readily observed on stimulating the peripheral ends of the cardiac nerves in the cardiac plexus, or the inferior cervical ganglion, the external or internal ansa, or the stel-

TABLE 1

Peripheral vago-sympathetic stimulation. Constrictor type of response. After atropine

ANIMAL	TEST NUMBER	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF CHANGE		
				Blood pressure	Heart rate	Volume of coronary flow
The right vagus						
		cm.	*seconds*			
4	15	8	30	1	0	−9
			60	−1	3	−20
4	16	6	15	−2	−3	0
			60	−3	0	−6
4	29	7	55	−4	0	−10
			85	−4	−4	−7
8	34	6	20	−4	26	−6
			60		8	−10
9	42	6	70	−2	0	−18
11	23	10	35	0	0	−6
12	20	8	45	−10	4	−16
15	26	6	35	−2	−3	−19
			75	−1	−5	−29
15	39	4	25	−4	−3	−15
			115	−4	−3	−20
16	41	6	35	4	−6	−7
17	39	6	30	3	0	−12
17	49	4	35	0	0	−14
18	14	6	10	−2	0	3
			45	−3	3	−4
22	27	6	15	−3	5	−6
			55	−8	0	−13
22	29	6	13	7	3	1
			60	−3	0	−7
25	25	6	30	−5	0	−11
			60	−5	0	−12
25	30	6	30	−6	4	−1
			60	10	3	−4
The left vagus						
5	39	6	15	−1	−9	4
			35	−5	−15	−10
5	40	4	55	−5	−4	−10
			80	−6	−4	−7
6	28	6	15	0	0	−9
			55	0	7	−11
8	35	6	20	0	−4	−1
			60	1	−1	−5
9	29	6	50	−4	−3	−7

TABLE 1—*Concluded*

ANIMAL	TEST NUMBER	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF CHANGE		
				Blood pressure	Heart rate	Volume of coronary flow
The left vagus—*Concluded*						
		cm.	*seconds*			
9	41	6	55	−4	0	−16
11	27	8	35	−3	0	−16
13	48	8	20	−3	−8	−6
14	41	4	20	−5	−7	−11
			55	−12	−9	−20
15	29	4	30	1	−5	−10
			60	−10	−5	−9
16	42	6	35	3	−6	−3
17	34	6	20	−3	0	−12
			45	−4	0	−18
17	40	6	35	0	0	−9
17	53	4	20	3	0	−14
22	22	6	45	−40	0	−15
25	23	6	20	7	3	−1
			60	−15	3	−23

late ganglion. If the stimulations are applied along the course of the cervical vago-sympathetic the reactive changes are inconsistent except on the hypothesis of mixed trunks, as will be shown in detail below.

Coronary constrictor type of response to peripheral cervical vago-sympathetic stimulation. The effects of peripheral cervical vago-sympathetic stimulation on coronary flow in the dog are very inconstant and non-predictable. As a rule stimulation produces a decrease of the coronary flow, as Porter (4), Maass (5), Wiggers (6), Morawitz and Zahn (7), and Anrep et al. (9) (10) have all observed. However, we find that some tests seem entirely negative, and others lead to an increase in coronary flow by coronary dilatation as reported by Martin (3). The significance of these variations appears on further analysis.

The degree of coronary constriction obtained in a particular animal and individual test varies with many factors. The coronary state, or degree of contraction or of relaxation at the beginning of the test, is the most important of all the factors determining the type and amount of vaso-motor change. A test applied during a coronary dilatation or state of relative atonia produces a greater volume of constrictor response and vice versa. The active constriction is therefore greater if the degree of relaxation of the vessels at the moment is greater. It follows that the observed percentages of coronary constrictions in a series of tests bear little or no constant quantitative relation to each other, either when compared by animals or by widely separated tests in the same animal. If tests follow

at too short intervals so that the constrictor effect of the first stimulation has not entirely passed off, then the second response with the same stimulus is quantitatively less, and the effects should be explained by the factor of summation in the efferent coronary mechanism. If the intensities of stimuli are taken into account and the above factors are avoided, then the successive reactions in response to stimuli of constant strengths seem more nearly equal.

When coronary constrictions occur they last from 30 seconds to 3 minutes and more. The longer constrictions follow more vigorous stimulations. The latent period, i.e., the time of onset of the reaction as it appears in the record varies from 5 to 8 seconds.[8] The amount of coronary constriction varies from within the limits of error of the method to as much as 25 per cent of the initial volume flow. On an average 10 to 20 per cent may be considered a pronounced coronary constriction from efferent cervical vago-sympathetic stimulation, see table 1.

Failure to demonstrate clear-cut peripheral coronary constrictor reactions from the cervical vago-sympathetic trunk does not mean the absence of constrictor neurones and impulses. The dilator reactions are present in neutralizing volume.

Coronary dilator type of response to peripheral cervical vago-sympathetic stimulation. Some dogs give repeated coronary dilatations upon peripheral cervical vago-sympathetic stimulation, table 2.

In the examples quoted above in table 2, the increase in coronary flow on stimulation of the peripheral cervical vago-sympathetic was at times quite pronounced. The coronary dilatation was usually, but not always, accompanied by cardiac acceleration. (See 19–48 and 21–32 for exceptions.) We believe that the rate and the dilator reactions are mediated by independent neurones, although the axones are in the common nerve trunk.

Anrep and Segall (10) have shown that neither change in heart rate nor stroke action have any influence, as such, on the minute volume of the coronary flow. We accept this view and are satisfied that the results presented here are not conditioned by simultaneous changes in heart rate.

[8] The latent period for cardiac inhibition is one second or less, for cardiac acceleration a trifle longer. But for coronary vessels, like other smooth muscle structures, the latent period is longer—from 5 to 8 seconds, not so great as 10 to 15 seconds recorded by Rein (18). Tabulated measurements at 10 seconds have been used freely to emphasize the earlier responses of heart rate. They consider only the initial coronary changes.

In an animal of sensitive reactive capacity the effects of an efferent coronary stimulation may set up changes that are acute and of comparatively short duration. The vessels do not necessarily stabilize at the preceding state of contraction but undergo a prolonged state of constriction or dilatation lasting several minutes. The vessels remain responsive to further stimulation during these vasomotor tone cycles but the per cent of change offers unusual variations.

TABLE 2

Peripheral vago-sympathetic stimulation. Dilator type of response. After atropine

ANIMAL	TEST NUMBER	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF CHANGE		
				Blood pressure	Heart rate	Volume of coronary flow
The right vagus						
		cm.	*seconds*			
2	27	5	20		8	31
3	7	6	20	−6	32	23
			40	−9	20	25
			60	−9	8	10
3	27	6	10	6	11	−3
			50	0	15	−8
3	9	6	10	1	8	12
			60	−12	0	0
5	22	6	10	4	3	−1
			60	8	3	3
5	31	6	10	3	8	8
			60	3	8	4
6	27	6	15	4	34	16
			55	20	11	0
7	18	6	10	0	−3	2
			50	2	3	0
9	40	6	60	14	3	20
12	23	8	35	4	4	5
19	43	6	20	−2	0	1
			40	−14	5	2
			60	−6	5	7
19	44	6	30	−1	9	10
			65	0	9	13
19	48	6	15	−3	0	15
			35	3	0	15
20	36	6	33	0	0	2
21	31	6	10	−2	20	0
			35	3	7	5
			75	3	7	6
The left vagus						
5	29	6	15	−1	0	6
			60	−1	0	−3
12	22	8	30	10	0	3
15	28	6	30	0	0	−2
			50	−4	−3	4
19	50	4	20	−4	0	5
			30	−3	−5	9
			60	0	−5	4
21	32	6	10	7	0	8
			50	5	0	6

They should be interpreted as clearly due to active vaso-dilatations of the coronary vessels.

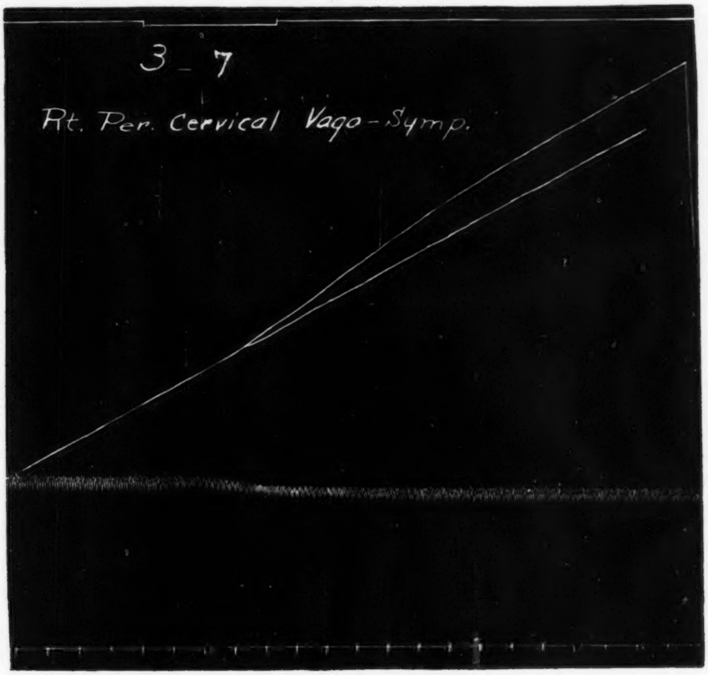

Fig. 2. Coronary dilatation upon stimulating the peripheral end of the right vago-sympathetic. Time 5 seconds.

Protocol: Dog 3, weight 6.5 K., amytal, ether, heparin, atropine.
Test 7: Stimulating the peripheral right cervical vago-sympathetic.

TIME	BLOOD PRESSURE	PER CENT CHANGE	HEART RATE	PER CENT CHANGE	CORONARY FLOW	PER CENT CHANGE
10″ Before	68		150		42	
10″ After	68	0	180	20	42	0
20″ After	64	−4	198	32	52	23
30″ After	63	−7	192	28	56	34
45″ After	62	−9	168	12	51	24
60″ After	62	−9	162	8	46	10
75″ After	61	−12	162	8	42	0
90″ After	61	−12	162	8	42	0

Similar relations exist between constrictor and dilator neurones in the left cervical vago-sympathetic trunk. Tables 1 and 2 give examples.

However, in 6–27 stimulation of the right nerve induced coronary dilatation while the left 6–28 induced constriction. This is an example of apparent unequal bilateral distribution of the dilator and constrictor fibers. In other animals the reaction is diphasic, a fact revealed by a series of measurements in successive intervals after the beginning of the stimulus (table 2).

It is important that coronary dilatation does not always accompany the acceleration of rate, and vice versa. Examples are included in the tables. Such instances indicate an overlapping and casual rather than a causal anatomical association as between the coronary dilator and cardiac accelerator nerve components of the cervical vago-sympathetic trunk. The variation of volume of acceleration in different cardiac nerves below the inferior cervical ganglion was first noted by Schmiedeberg. We have observed a similar variability in volume of reaction of the coronary pathways.

Cardiac nerves below the inferior cervical ganglion exhibit this type of coronary reactive variation by what may be described as unequal mass reaction, revealed by the variations in per cent of change.

The observed differences rest on two types of variability. In the one type there is a variable content of neurones of one or the other group giving rise to a larger or smaller coronary change on stimulation. In the other type both constrictor and dilator neurones are present as revealed by removing the function of one or the other. Below the inferior cervical ganglion coronary dilatation is the preponderant reaction of all the mixed cardiac nerves. The neutralizing presence of coronary constrictors is revealed only by any unexpectedly small increase in the coronary flow.

Coronary response to peripheral cervical vago-sympathetic stimulation is the resultant effect of simultaneous stimulation of coronary constrictors and coronary dilators. If coronary constrictor and coronary dilator neurones are both present in the cervical vago-sympathetic, then both are simultaneously stimulated and the coronary reaction is the algebraic sum of the two antagonistic influences. The large number of adequate tests giving only a slight per cent of change is fully accounted for by this explanation.

The diphasic responses reported in table 2 are satisfactorily explained when it is understood that antagonistic nerve units are being stimulated. In 5–39 and 5–40, the weaker stimulation induced an initial dilatation followed by a constriction, the stronger stimulation produced an immediate constriction. Many disconcerting and seemingly contradictory coronary responses to cervical vago-sympathetic stimulation and to reflex stimulation are harmonized by this concept of composite nerve stimulation.

Experiments are also in progress, in search of confirmatory evidence of the exact origin and course of the efferent fibers of each component of the cervical vago-sympathetic responsible for the coronary dilatations and con-

strictions. At present it is assumed that the dilator reactions are due to connecting thoraco-sympathetic neuronal chains which reach the coronaries by indirect routes above the inferior cervical ganglion, presumably the middle and superior cervical ganglia, with synaptic connections in these ganglia.

Only Morawitz and Zahn of those reporting in the literature questioned Porter's original deduction that the coronary constrictor fibers of the cervical vago-sympathetic are true efferent vagal neurones. Morawitz and Zahn regarded the conclusive proofs of vagal origin of coronary constrictor neurones as yet to be made. Later authors, without further evidence, accept the view of vagal origin.

The coronary dilators in the cervical vago-sympathetic have been observed by Martin, and by Morawitz and Zahn. The hypothesis of thoracic origin of their preganglionic neurones has been unhesitatingly accepted. The correct determination of the central origin of both sets of coronary nerves can be secured only by methods that remove one or the other sets from the physiological field. Preliminary results of experiments to answer the problem by the method of degeneration of the efferent neurones of the vagus trunk have been announced by us before the American Physiological Society (11).

Coronary response to stimulation of the stellate ganglion and of nerves contributing directly to the cardiac plexus. Maass (5) demonstrated the presence of coronary dilators by stimulating branches from the stellate ganglion to the cardiac plexus. All later experimental evidence confirms this as the main and most direct channel of coronary dilator neurones from their spinal origin to the arterial terminations. The dilator neurones are apparently in greater numbers in this central area of the cardiac outflow, judged by the ease of stimulation and the volume of dilator response.

There is a great variation in the gross anatomical pattern of nerve bundles condensed into and originating from the stellate and the ansal ring. This is true in respect to the exact origin and relative size of the two connectors of the ansa to the inferior cervical ganglion; and in the number, relative size, and exact origin in each particular animal of the cardiac nerve filaments along the course of the vago-sympathetic trunk contributory to the cardiac plexus.

It is interesting that Schmiedeberg (19) in 1871 correctly observed and figured the gross anatomical relations of these branches to the cardiac plexus, as did Pavlov (20) in 1887. Schmiedeberg's description of the origin and course of cardiac accelerator nerves still represents an accurate anatomical picture. In his figure the superior cardiac nerve of the dog is shown arising from the inferior cervical ganglion, and the inferior cardiac nerve from lower down the trunk of the vago-sympathetic. The variations of this plan that are of significance are: 1, either or both cardiac

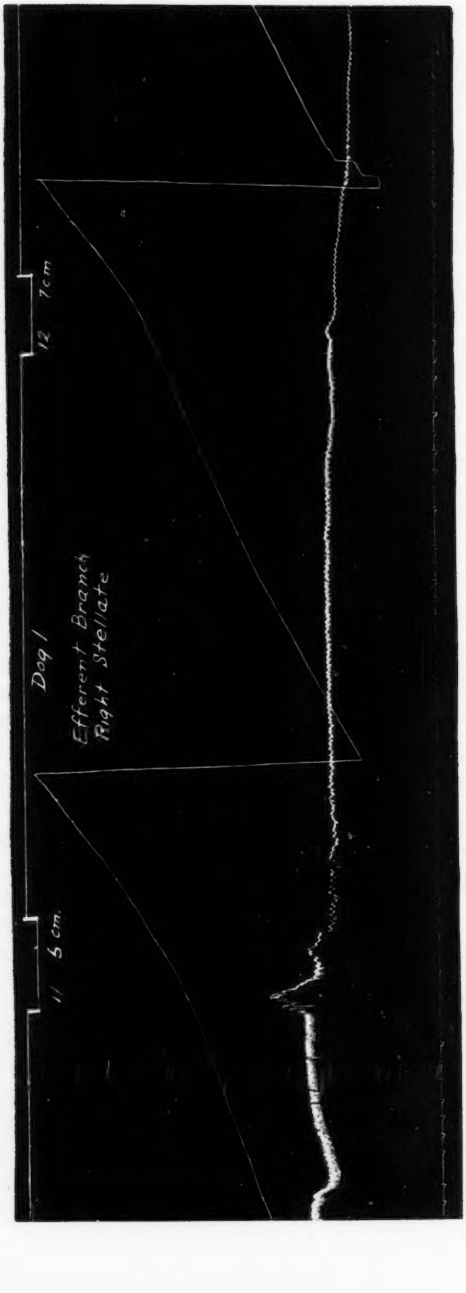

Fig. 3. Two successive stimulations of the stellate and internal ansa, freed from the spinal cord. Test 11, 6 cm., test 12, 7 cm.

nerves may be represented by two or more filaments; 2, the superior cardiac nerve may arise independently from the ventral face of the inferior cervical ganglion, or in common with the recurrent laryngeal, from which it separates after a course of 6 to 12 mm.; 3, the inferior cardiac nerve may arise *a*, from the internal ansa; *b*, from the vago-sympathetic trunk below the union of the internal ansa with the vago-sympathetic, or *c*, from the trunk above the union of the vagus and the internal ansa and nearer

Protocol: Dog 1, weight 7 K., morphine and ether, heparin. Blood pressure from the right carotid. Coronary flow from the coronary sinus. Coronary blood returned via the femoral vein. Unrestrained arterial pressure.

TIME	BLOOD PRESSURE	PER CENT CHANGE	HEART RATE	PER CENT CHANGE	CORONARY FLOW	PER CENT CHANGE
Test 11: Peripheral stimulation of right stellate, freed from the spinal cord, 6 cm.						
10″ Before	78		143		23	
10″ After	71	−8	201	41	37	61
20″ After	71	−8	221	54	40	74
30″ After	60	−23	224	66	42	83
45″ After	60	−23	205	43	50	117
60″ After	62	−20	195	37	49	113
90″ After	63	−19	186	30	34	48
120″ After	64	−18	186	30	31	35
150″ After	63	−19	176	23	31	35
Test 12: Peripheral stimulation of right stellate, 7 cm.						
10″ Before	60		176		28	(22)*
10″ After	59	−2	238	35	Beginning to increase	
20″ After	59	−2	238	35	45	61 (96)
30″ After	58	−3	227	29	50	79 (117)
40″ After	55	−8	220	25	49	75 (113)
60″ After	50	−17	208	18	34	21 (48)
90″ After	48	−20	189	7	32	14 (39)

* Figures in brackets are computed in per cent of change of flow from the coronary sinus at the beginning of test 11.

the inferior cervical ganglion. If the nerve is represented by several filaments they may arise from two or more of these sources.

The coronary reactions to stimulation of these several regions is briefly presented as follows:

Coronary reactions to stimulation of the stellate ganglion. Stimulation of the stellate ganglion isolated from the cord by sectioning the rami of the connecting thoracic spinal nerves produces prompt and massive coronary dilatation. The stellate is beyond question the condensed pathway for the great outflow of coronary dilators from the thoracic cord to the cardiac plexus.

Coronary reactions to stimulation of the internal ansa. Stimulation of the internal ansa between the stellate and the vago-sympathetic trunk also produces profound coronary dilatation. The dilatation amounts to as much as 65 per cent to 120 per cent or more. It is the most reactive path and undoubtedly contains the majority of coronary dilator nerve units linking the spinal cord with the coronary arteries.

If the direct or continuation branches of the internal ansa, or of the lower vago-sympathetic, or the inferior cardiac nerve itself are stimulated the reaction is somewhat less than that of the internal ansa. This implies a spread of axones through the smaller nerve filaments toward and into the cardiac plexus.

Coronary reactions to stimulation of the external ansa. The external ansa varies greatly in relative development from a strong trunk in some dogs to the slightest filament in others. In the former type the response to stimulation is a pronounced coronary dilatation, but in the latter type dilatation is slight and insignificant. Seldom does the total dilatation approach the volume of that produced through the internal ansa, due to dearth of coronary neurones.

After nicotine poisoning of the inferior cervical ganglion the reaction of the coronaries to external ansal stimulation is nil, indicating that this connective contains only preganglionic axones, which have passed through the stellate to synapses in the region of the inferior cervical ganglion.

Stimulation of the superior cardiac nerve. The superior cardiac nerve of the dog has long been known as the pathway of efferent rate controlling, i.e. of inhibitor and cardiac accelerator neurones. It is of equal importance in the set-up of coronary dilator neurones. Its stimulation yields coronary dilatation in unexpectedly variable percentages, see tables.

When the superior cardiac nerve is well developed, its stimulation may produce coronary constriction, as shown in figure 4. Diametrically opposed coronary reactions admit only one interpretation, namely, that the superior coronary nerve contains both coronary dilator and constrictor neurones. Inhibitory fibers also occur in this branch and are active in the normal nerve, but after atropine only cardiac acceleration with either coronary dilatation or constriction occurs. It is the chief pathway of coronary constrictor neurones from the vagus to the heart. It is clear that the type of coronary reaction depends upon the dominant mass of neurones represented in the nerve.

Stimulation of the inferior cardiac nerve. Extreme coronary dilatation follows stimulation of the inferior cardiac nerve, or of its filaments when they are present as multiple branches. The dilator response is clear-cut, follows promptly after a short latent period, and is of the type that suggests uncomplicated peripheral dilator stimulation.

We have not observed primary constrictions from nerves this low in

the group of cardiac nerve branches, though figure 4 shows constriction in the after-period. If constrictor neurones are present, their reactive influence is neutralized by the greater volume response of dilator neurones.

A further point of comparison is that the total volume of the dilator response as between the superior and the inferior cardiac nerves is strongly

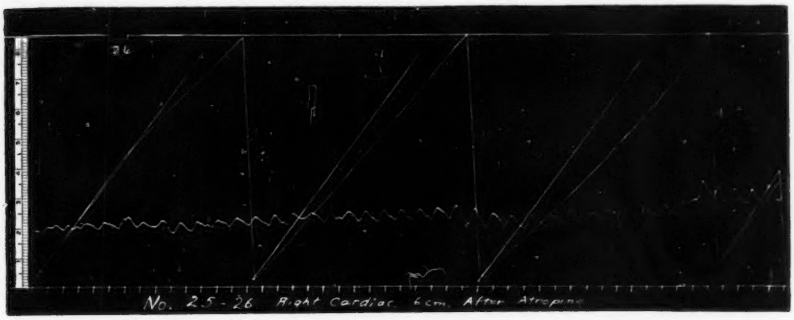

Fig. 4. Stimulating right superior cardiac nerve, 6 cm.

Protocol: Dog 25, weight 5.1 K., heparin 60 mgm. per K., atropine. Uncontrolled blood pressure from right carotid, coronary flow from the coronary sinus; time, –5 seconds.

Test 26: Stimulation of the right superior cardiac nerve, 6 cm. The coronary constriction occurs in the presence of an antagonistic rise of blood pressure and lasts into the 4th minute. The heart rate remains constant. The experiment was repeated in test 33 with similar reaction.

TIME	BLOOD PRESSURE	PER CENT CHANGE	HEART RATE	PER CENT CHANGE	CORONARY FLOW	PER CENT CHANGE
10″ Before	40		198		82	
15″ After	41	2	204	3	64	–22
30″ After	42	5	204	3	60	–27
60″ After	46	15	201	1	66	–19
100″ After	48	20	192	–3	70	–15
140″ After	44	10	198	0	71	–13
180″ After	49	22	198	0	73	–11
210″ After	59	47	198	0	94	15

in favor of the latter. This indicates a greater number of coronary dilator neurones flows through the internal ansa, and the more direct paths of the cardiac plexus to the coronaries.

Coronary dilator reactions through lower dorsal pathways. It has been shown above that the autonomic spread of efferent coronary dilator nerves extends as far in the cephalic direction as the superior cervical sympathetic ganglion. Apparently the preganglionic neurones form synapses in the

stellate, the inferior cervical and the superior cervical ganglia. They spread like a great fan over this wide area of sympathetic ganglia, yet

TABLE 3

*Peripheral stimulation of the superior cardiac nerve**

ANIMAL	TEST NUMBER	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF CHANGE		
				Blood pressure	Heart rate	Volume of coronary flow
		cm.	*seconds*			
10	20	10	15	2	0	2
			45	3	0	−12
10	21	8	20	3	Irreg.	8
			60	6	0	4
47	22	6	10	6	−4	−9
			60	14	−4	−20
10	23	6	10	0	−3	−7
			60	−3	0	−3
10	24	4	10	2	0	17
			60	−3	4	12
12	41	10	30	−1	65	18
			70	−5	13	−2
12	42	8	20	−2	66	12
			65	−10	15	−11
12	43	6	30	6	60	32
			70	−1	8	8
12	44	4	25	3	58	28
			70	−8	4	−8
13	42	8	20	0	46	−2
			70	0	4	−1
13	49	8	20	−3	48	−4
21	27	6	15	3	3	7
			40	0	3	9
24	38	4	15	−2	32	−8
			70	−1	0	−13
25	26	6	15	3	3	−22
			60	14	2	−19

* A comparison of the data of table 3 reveals incidentally differences of mass distribution of coronary nerves and accelerator nerves. For example, animals 12, 13 and 24 have a large mass of cardiac accelerators in the superior coronary nerve while animals 10 and 25 have almost no accelerators but both yield vigorous coronary constrictions. Animal 24 on the other hand yields a 13 per cent decrease in coronary flow, while animal 12 yields a 32 per cent increase in coronary flow. Obviously, the nerve influences on volume of coronary flow and on heart rate are independent functions.

the relative volume of dilator reaction and number of neurones is greatest in the more direct paths from the stellate through the cardiac nerves or their subdivisions to the cardiac plexus.

The question arises whether or not a similar spread of coronary nerves exists through the thoracic sympathetic chain in the caudal direction, as shown by Cannon and co-workers (21) for the cardiac accelerator outflow

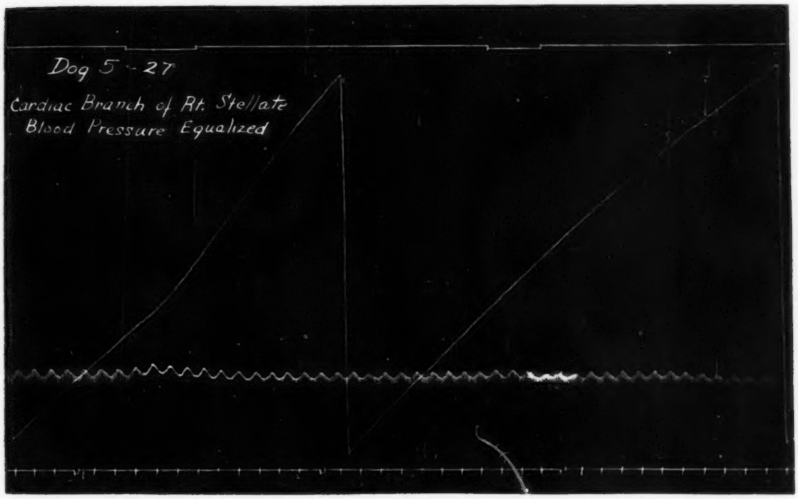

Fig. 5. Coronary dilatation upon stimulating the peripheral end of the internal ansa and cardiac branch from the right stellate, after atropine. Blood pressure equalized 6 cm.

Protocol: Dog 5, weight 10.5 K., ether, morphine, heparin, and atropine. Both vagi cut, arterial pressure equalized.

Test 27: Stimulation of the peripheral end of the cardiac branch from the right stellate through the internal ansa, 6 cm.

TIME	BLOOD PRESSURE	PERCENT CHANGE	HEART RATE	PER CENT CHANGE	CORONARY FLOW	PER CENT CHANGE
10″ Before	57		162		66.0	
15″ After	62	9	270	66	82.0	24
30″ After	56	−2	162	0	99.0	50
60″ After	56	−2	186	15	91.0	39
80″ After	56	−2	168	4	77.0	16
100″ After	57	0	156	−4	66.0	0
140″ After	53	−6	162	0	57.0	−14
230″ After	56	−2	162	0	56.0	−16

in the cat. There are no data in the literature describing coronary nerves in the lower thoracic pathways. The presence of cardiac accelerators has been noted by several investigators.

Perman (22) has described cardiac nerves arising from thoracic ganglia as low as the 6th thoracic ganglion in the sheep and goat. He presents illustrations of their composition and course along the azygos veins, his figures 7 and 11. Ionesco and Enachesco (23), reporting from Danielopolu's clinic at Bucharest, note the larger size of the nerves from the left thorax in the calf, their figure 1. They also describe cardiac branches in the left thorax of the dog. Ionesco and Enachesco demonstrated the presence of cardiac accelerators in the thoracic nerves of the sheep and goat. A further demonstration by Ionesco and colleagues (24) is of the greatest significance, namely, that these lower thoracic nerves contain afferent neurones which upon stimulation bring about reflex cardiac acceleration.

We have observed very delicate thoracic nerve filaments from the 4th, 5th and 6th thoracic ganglia in the dog, that extend outside the pleura and into the mediastinal tissue. These are extremely minute on the right, but on the left are somewhat larger. We have noted in dogs that the largest of these nerves is from the left 6th thoracic sympathetic ganglion. Occasionally this nerve is joined by a small branch from the 5th, the two forming a nerve that can be separated with fair ease.

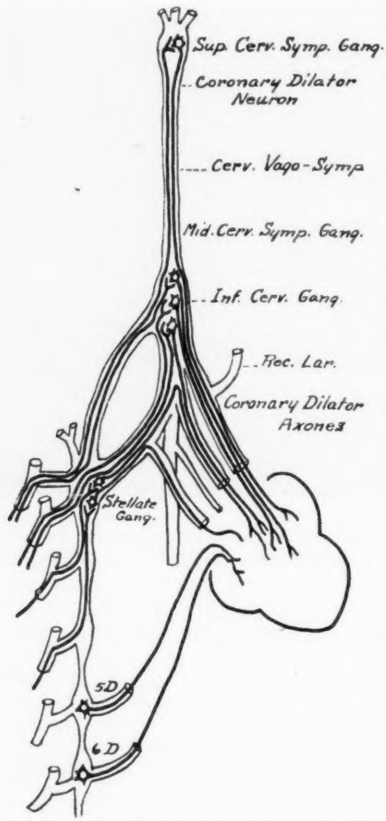

Fig. 6. Diagrammatic representation of the efferent pathways of the coronary dilator neurones from cardiac through the sympathetic ganglia to the coronary vessels. The connections between the spinal cord and the lower thoracic post-ganglionic neurones have not been established.

We have successful physiological tests on a somewhat weakened animal, yet the importance of positive results justifies a report without delaying for further data. Stimulation of the small filament arising from the right 5th thoracic sympathetic ganglion at 6 cm. of the induction coil produced coronary dilatation. The animal was under atropine, the cervical vagi were cut and the right accelerators were cut distal to the stellate.

The per cent of change in the blood pressure was slight, but the coronary flow increased 12 per cent. Incidentally, the table shows a cardiac acceleration equivalent to 22 per cent.

Stimulation of the branches of the left 6th thoracic ganglion resulted in a still more pronounced coronary dilatation, the maximum being a 69 per cent increase in coronary flow. The stimulus was stronger, 4 cm., which was vigorous for this type of test. The changes of blood pressure were moderate, 7 per cent, but the cardiac acceleration was greater. The heart and the coronary vessels were very sensitive to accelerator and to dilator coronary nerves. The accelerator influences disappeared more rapidly than the coronary dilator reaction.

TABLE 4

Dog 14; weight 7.25 K., heparin, atropine. Right accelerators and right and left vagi cut. Heart somewhat weakened, but still very sensitive to nerve stimulation

Tests: Stimulation of thoracic sympathetic branches to the cardiac plexus arising below the stellate ganglion.

ANIMAL	TEST NUMBER	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF INCREASE IN		
				Blood pressure	Heart rate	Volume of coronary flow
		cm.	*seconds*			
14	44*	6	30	3.0	22.0	12.0
	46**	4	20	7.0	100	69.0
			50	7.0	50	26.0

* Stimulation of small branch from the right 5th thoracic ganglion to the mediastinum.

** Stimulation of a branch from the left 6th thoracic ganglion.

Coronary dilator autonomic pathways spread through the thoracic chain in the caudal direction at least to the 6th thoracic ganglion.

SUMMARY

The data and discussions presented in this paper are condensed and briefed from a very large mass of observations on the dog. The illustrations are chosen for types and the protocols with each illustrate the method of comparison used. The major points are:

1. The efferent neurones to the coronary vessels are of the usual vascular antagonistic types of constrictors and dilators.

2. The coronary dilator neurones are of greater mass development, produce the more profound coronary reactions, and are more sensitive to stimulation.

3. The coronary dilator neurones are of thoracic spinal origin. They reach the heart chiefly via the stellate and the inferior cervical ganglia and the cardiac nerves arising from this region.

4. Some dilator nerves are found as far cephalad as the superior cervical ganglion and as far caudad as the 6th thoracic ganglion. These extreme limits of the spread of autonomic dilator nerves to the cardiac vessels are new demonstrations.

5. The coronary dilator nerves and the cardiac accelerator nerves reach the heart via the nerves of the cardiac plexus. This is an overlap of pathway and the neurones are not equally present in any particular cardiac nerve.

6. It is suggested that the extreme cephalic margin of the coronary dilator outflow is a remnant of phylogenetic history and of embryonic development.

7. Coronary constrictor nerves are distributed to the heart via the cervical vago-sympathetic, the inferior cervical ganglion and the nerves of the cardiac plexus.

8. Stimulation of mixed cardiac trunks containing both coronary dilator and constrictor neurones yields results that are unpredictable, since the physiological response is the algebraic sum of the two influences imposed on the coronaries. The cervical vago-sympathetic of the dog is also of this type and its stimulation yields coronary constriction or dilatation in proportion to the relative mass development of the two classes of neurones in the mixed trunk.

9. The cervical vago-sympathetic of the dog is a common nerve trunk in one sheath from which the constituent neurones cannot be separated by anatomical means. This has resulted in great confusion in the interpretation of physiological experimental results beginning with Martin's (2) first report on the presence of coronary nerves.

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THE EFFECT OF TEMPERATURE ON THE RATE OF BLOOD FLOW IN THE NORMAL AND IN THE SYMPATHECTOMIZED HAND

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The qualitative effect of changes in local temperature upon the circulation in the human extremities is generally known. Cold brings about a decrease while heat increases the rate of flow. These facts have been demonstrated by direct measurements of the volume flow of blood (Hewlett, Van Zwaluwenburg and Marshall, 14). They have also been inferred from measurements of the rate of loss of calories (Stewart, 27). No exact quantitative relationship, however, has been established between the local temperature and the rate of blood flow in the human extremity. By means of a study of the circulation in sympathectomized as well as in normal extremities, an attempt has been made to elucidate the mechanism of the local thermal control of the peripheral circulation.

METHOD. The rate of blood flow was determined in the hand in these experiments by a modification of the method first described by Hewlett and Van Zwaluwenburg (13) of recording the rate of increase in the arm volume when the venous outflow is occluded. With a "collecting pressure" below diastolic blood pressure, they observed no hindrance to the arterial inflow. They employed an air plethysmograph and made no attempt to control the temperature of the arm. In later experiments (Hewlett, Van Zwaluwenburg and Marshall, 14), the local temperature was changed by immersing the arm in water in the plethysmograph. The temperature of the water was then varied by circulating hot or cold water through a metal tube, running through the water in the plethysmograph. No measurements were made of the quantitative relationship between different controlled temperatures and the rate of blood flow. Their technique was accordingly modified so that the temperature was controlled by immersion of the hand in water in an insulated calorimeter.

A metal cylinder 5 inches in diameter and 12 inches long is fitted inside a 6 inch cylinder 13 inches long, and the space between the two containers is insulated. Through one end are inserted a stirring propeller and 7.5 watt heating lamp. On the upper surface are openings for a thermometer and for rubber tubing connected to a Brodie bellows of 10 cc. capacity.

A wire mesh platform to support the hand lies 1 inch from the bottom of the plethysmograph. The hand is inserted to the wrist through a rubber membrane having a cuff which fits lightly so as not to constrict the superficial veins. The cuff is attached to the skin with rubber cement. The rubber membrane is then stretched over the opening of the inner metal tube and wedged on by a metal ring with a flanged edge. A pad of felt ½ inch thick is adjusted to the wrist and is held in place by an iris diaphragm. Proximal to the joint is wrapped a pressure cuff 1.5 inches in width. This cuff is inflated from a 2-liter bottle fitted with a pressure gauge. With the hand at rest on the mesh platform, the plethysmograph is ready to be filled with water at the desired temperature. When it is full, 20 cc. of water are aspirated in order to allow for air transmission to the Brodie bellows.

The patient reclined quietly for a half-hour before determinations were made. The hand was approximately at the level of the heart. Between 5 and 15 minutes were required for the blood flow to become stable with the hand in water at any given temperature (5). When the temperature of the bath was changed, it was necessary to allow sufficient time for stability to be reached.

It was soon found that the blood flow was markedly reduced by emotional or painful stimuli. Loud noises, interruptions, embarrassment, and discomfort were therefore avoided. For experiments of long duration, or for those in which the same patient was to be observed on different days, basal fasting conditions were found to be necessary. Careful notes were made of the room temperature, mouth temperature, and arterial blood pressure.

The rate of blood flow was not constant for any length of time. Rhythmic fluctuations, sometimes of great magnitude, took place, especially in certain subjects. Fluctuations in blood pressure records not associated with the pulse or respiratory cycle were first described by Traube (29) and by Hering (12). Concomitant fluctuations in leg volume were shown by Bayliss and Bradford (4) to depend upon variations in vasomotor tonus, since they disappeared after cutting the vasomotor nerves. The rhythmic fluctuations in blood flow found by Hewlett and Van Zwaluwenburg (13) were ascribed to the same mechanism. Figure 1 presents an original record of blood-flow determinations taken in both hands at the same time. The Traube-Hering waves are seen to be simultaneous and of approximately equal magnitude on both sides of the body. After section of the sympathetic nerves by cervico-dorsal ganglionectomy (33), these waves disappeared as shown in figure 2.

Variations in blood flow, which may amount to as much as 200 per cent in certain subjects, make it essential to use caution in the interpretation of results. In order to minimize the error, usually 5 consecutive deter-

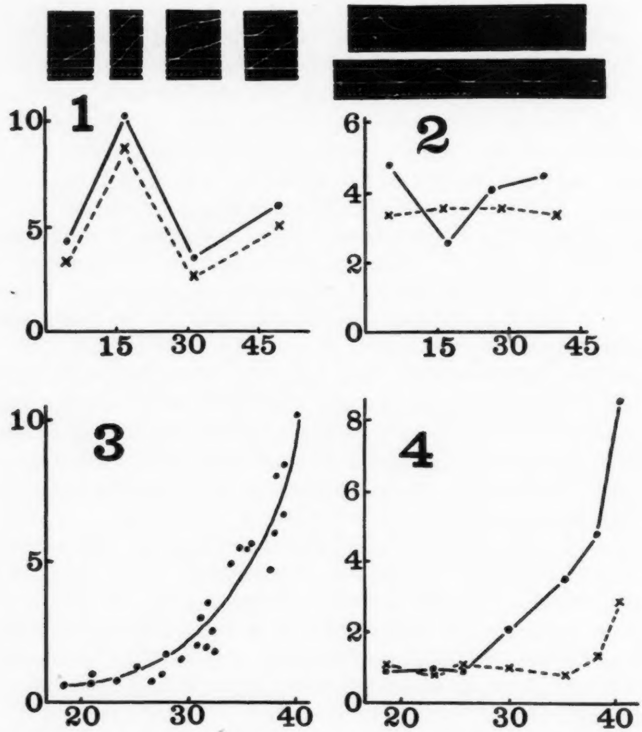


Fig. 1. Blood flow determinations in both hands simultaneously to show the Traube-Hering waves. In this as in the following figures ordinates represent cubic centimeters of blood flow per 100 cc. hand volume per minute. Abscissae, time in seconds. Tracing: upper curve left, lower curve right hand. Chart: solid line right, interrupted line left hand.

Fig. 2. Blood flow determinations to show that Traube-Hering waves are absent in sympathectomized hand. Tracing: upper curve, left hand, normal; lower curve, right hand, sympathectomized. Chart: solid line, normal; interrupted line sympathectomized hand.

Fig. 3. Effect of changes in local temperature on the rate of blood flow in the normal hand; twenty-five determinations on seven different days under basal fasting conditions. Ordinates, blood flow; abscissae, temperature, degrees Centigrade.

Fig. 4. Effect of changes of local temperature in one hand on rate of circulation determined simultaneously in both hands. Solid line, blood flow in left hand at temperatures indicated along abscissae. Interrupted line, blood flow in right hand kept at constant temperature 23 ±0.2 degrees Centigrade.

minations were made and the mean was selected. In some individuals it was impossible to obtain consistent readings.

The accuracy of the volumetric method was considered by Hewlett

and Van Zwaluwenburg (13) to be approximately 20 per cent in favorable cases. When observations were made at different times on the same individual, variations of 50 per cent were not uncommon, while variations of 100 per cent or even more might occur. In this series of experiments with the temperature of the hand carefully controlled the results have generally been more consistent. The curve in figure 3 is compiled from 25 blood-flow determinations made on 7 different days on the same patient under basal fasting conditions. The maximal variation from the mean of any single determination was 40 per cent, but the curve obtained from day to day maintained this characteristic form.

Results. *Effect of local temperature on rate of blood flow.* As the temperature of the bath in which the hand was immersed was gradually increased, there followed a progressive rise in the rate of blood flow which could be expressed in the form of a curve (fig. 3). A similar curve for the effect of temperature on blood flow was always obtained, although the slope of the curve varied from one subject to the next. The curve is similar in shape to that described by Lewis (17) for the effect of temperature on the digital pulse volume.

This influence of temperature is primarily a local phenomenon. The flow in one hand, at constant temperature, was not influenced by the progressive increase in flow which accompanied the rise in temperature of the other hand. An example is illustrated in figure 4. The blood flow was determined in both hands simultaneously. The temperature of the right hand was kept constant at 23°C., while the temperature of the left hand was raised from 18° to 41°C. The characteristic increase of blood flow occurred in the left hand with no change in the flow of the right until the temperature of the left exceeded that of the body. At that point certain vasomotor reactions were probably initiated. These will be discussed below.

The mechanism which controlled the changes in blood flow with variations in local temperature was then sought.

Reflex changes of blood flow in the hand as a result of thermal stimuli applied to the body. In spite of the independence of the hands in relation to local temperature which has just been described, it is recognized that the application of cold or heat to one part of the body may be reflected in vasomotor changes in other regions. Pickering (24) has given an excellent review of the development of our knowledge on this subject.

Figure 5 illustrates the decrease in the blood flow of the normal hand when ice-bags were applied to the chest. This finding corroborates the observations of previous investigators.

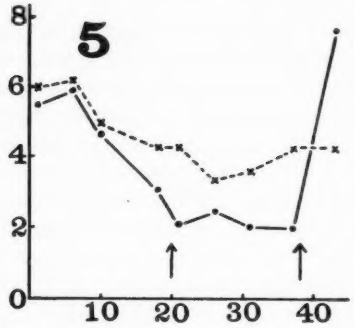
It has been established that this reflex effect of cold is mediated through the sympathetic vasoconstrictor nerves. Consequently the reflex should be abolished by removal of these nerves. The left hand of the patient

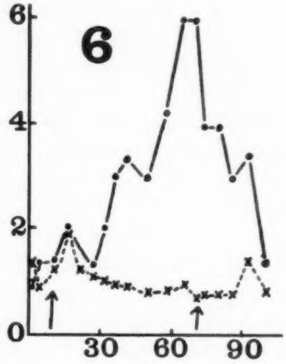
whose reactions are presented in figure 5 had been completely sympathectomized 2 years previously, because of intractable pain in the head, neck and shoulder. The completeness of the sympathectomy was carefully verified (32). In spite of the absence of all demonstrable sympathetic nerves, the blood flow in the left hand showed a definite decrease when cold was applied to the body (see fig. 5). It was interesting to note the reduction of the blood flow in both the normal and the sympathectomized hands from the expectation of unpleasant cold stimuli. Possibly mild apprehension resulted from the preparation of the ice-bags in the room. Thomas (28) and Lewis and Landis (19) have called attention to the drop in skin temperature of the sympathectomized hand when the patient was exposed to a cold environment. They have not attempted to explain the phenomenon. This vasoconstriction can be explained on the basis of adrenal secretion. The blood vessels in man become more sensitive to injected adrenalin some days after sympathectomy (9). Vessels thus sensitized also react to adrenine secreted in response to insulin hypoglycemia (9). Since in animals an increase in adrenal secretion occurs on exposure to cold (6), it is logical to expect that a similar reaction to cold should also take place in humans. The reduction of blood flow in the sympathectomized hand illustrated in figure 5 was therefore probably the result of adrenal secretion in response to cold.

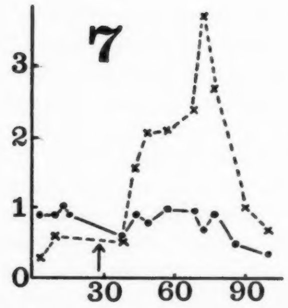
The effect of heat applied to the body on the rate of blood flow in the hand kept at constant temperature is depicted in figure 6. A six-fold increase in the circulation resulted from the reflex vasodilatation. The rise in blood flow in the left hand shown in figure 4, when the temperature of the right hand exceeded that of the body, is an additional example of this reaction (cf. Gibbon and Landis, 10). It has been shown that this vasodilatation depends on the integrity of the sympathetic nerves (19, 20). The absence of any increase in blood flow in the sympathectomized hand (see fig. 6) when the body was warmed confirms the observations of previous investigators.

Mechanism through which local temperature affects blood flow. Since it is understood that reflex changes in blood flow are mediated through sympathetic vasomotor nerves, it seemed possible that the increase in the rate of circulation which accompanied a rise in local temperature (see figs. 3 and 4) might also be controlled through these same nerves. If this were so, then paralysis of these nerves should be followed by a stable blood flow independent of local temperature. The surface temperature has been shown to rise after anesthesia of the peripheral nerves (30), after spinal anesthesia (23) or after injection of novocaine into the paravertebral sympathetic ganglia (31).

The effect of anesthetization of the sympathetic nerves on the rate of blood flow in the hand at constant temperature was therefore determined.







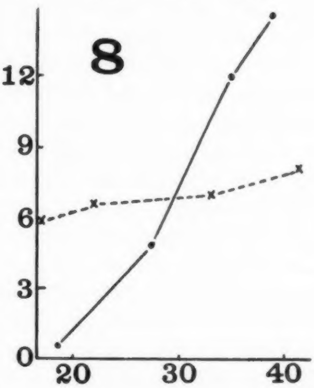


Fig. 5. Reflex effect of cold applied to the body on the rate of blood flow in the normal and in the sympathectomized hand. Both hands maintained at constant temperature 31.5 ± 0.5 degrees Centigrade. Solid line, normal; interrupted line, sympathectomized hand. Between the arrows ice bags were applied to both supraclavicular regions. Ordinates, blood flow; abscissae, time in minutes.

Fig. 6. Effect of applying heat to the body on the rate of blood flow in the normal in contrast to the sympathectomized hand. Both hands at constant temperature 19.0 ± 1 degrees Centigrade. Between the arrows a heated cabinet was placed over the body. Solid line, normal; interrupted line, sympathectomized hand. Ordinates, blood flow; abscissae, time in minutes.

Fig. 7. Effect of paralyzing sympathetic nerves on the rate of blood flow to the hand immersed in cold water. The arrow indicates the time of the novocaine injection. Interrupted line, left hand, injected; solid line, right hand, control. Temperature on both sides maintained at 17.5 ± 2 degrees Centigrade. Ordinates, blood flow; abscissae, time in minutes.

Fig. 8. Effect of changes in local temperature on the rate of circu'ation of the nomal hand in comparison with that of the opposite hand 10 days after sympathectomy. Solid line, left hand, normal; interrupted line, right hand, sympathectomized. Ordinates, blood flow; abscissae, temperature, degrees Centigrade.

The patient was seated before a table with both hands immersed in cold water (17.5 ± 2°C.) in the plethysmographs. Novocaine (1 per cent) was then injected into the region of the left inferior cervical and stellate ganglia. Within 20 minutes the patient had developed a Horner's syndrome and flushing of the left side of the face. As figure 7 shows, there was a concomitant rise in the rate of blood flow on the injected side from 0.5 to a maximum of 3.5 cc. per minute. As the anesthesia receded the blood flow returned to its basal level. It seemed clear therefore that the reduced rate of blood flow in the hand when immersed in cold water was the result of tonic vasoconstrictor impulses travelling over sympathetic pathways.

To test this hypothesis further, experiments were done to determine whether the rate of blood flow in a hand deprived of sympathetic nerves would remain stable and unaffected by changes in temperature. The rate of blood flow was determined in both hands of a patient 10 days after removal of the right cervicodorsal sympathetic nerves from the inferior cervical through the second thoracic ganglion. Figure 8 gives the results obtained in this experiment. The change in rate of blood flow in the normal hand with increases in the temperature of the bath was characteristic. On the sympathectomized side the rate of blood flow varied only slightly. Neither the low rate in the cold nor the high rate in the hot water was present as on the normally innervated side.

Six months later the blood flow in the hands of the same patient was investigated. The completeness of the sympathectomy was confirmed by appropriate tests (32). On immersion of the sympathectomized hand in cold water the blood flow dropped to a low level (see fig. 9). Whereas it had seemed clear before that the variation of blood flow in response to changes in local temperature was mediated through the sympathetic nerves, now in the absence of any demonstrable sympathetic nervous impulses, wide variations in the rate of blood flow were again apparent with changes in the local temperature. This observation has been confirmed in 8 other patients several months after sympathectomy.

Close inspection of the curves which are characteristic for the effect of temperature on the blood flow through the normal (see figs. 3 and 4) and through the sympathectomized hand (see fig. 9) revealed that they were of the same general shape. The chief difference in the two curves lay in the fact that the extremes of constriction and of dilatation were absent in the sympathectomized hand. It is probable that reflex vasoconstriction, except that from adrenal secretion (see fig. 5), and reflex vasodilatation (see fig. 6), are absent after sympathectomy. The difference in the curves obtained for the effect of local temperature on the circulation in the normal in contrast to that of the sympathectomized hand could be explained on the basis of the absence of reflex vasomotor effects. The mechanism of the purely local effect of temperature would therefore not

be mediated through the sympathetic nerves. The possibility was considered that variations in adrenal secretion might have accounted for the changes in circulation. However, in further experiments on a patient with both hands sympathectomized, changes in the local circulation were dependent only on the local temperature. The blood flow in the warm hand was rapid. At the same time, the other hand, immersed in cold water, showed a slow circulation.

Loss of tone (11) might explain the steady rate of flow immediately after sympathectomy (see fig. 8), but if so it does not explain the progressive increase in blood flow with rise in local temperature found in the hand 6 months after sympathectomy (see fig. 9).

The changes in circulation might also be explained on the basis of the direct effect of temperature on the calibre of the blood vessels (15), or on the viscosity of the blood (7). Still other reflexes of a vasomotor nature, for example the posterior root vasodilators (3, 8), or the axon reflex (2), might account for the changes. It is also conceivable that heat might increase the production of an H-substance, according to the theory advanced by Lewis (16). All of these hypotheses will be considered in the discussion.

Since it is well recognized that temperature has a direct effect upon the speed of chemical processes, the possibility was entertained that the increase in blood flow associated with a rise in the temperature of the hand might be based on a chemical phenomenon.

Quantitative relationship between local temperature and blood flow. The fundamental laws for the influence of temperature upon the speed of chemical reactions were developed from empirical observations by Arrhenius (1). His equations demonstrate that a straight line results when the logarithm of the rate is plotted along the ordinates and the reciprocal of the absolute temperature is plotted along the abscissae.

The curve which illustrates the effect of temperature on the rate of blood flow in the hand six months after sympathectomy (see fig. 9) was accordingly plotted by the Arrhenius equation. As can be seen in figure 10, the points lay on a straight line. This relationship between circulation and temperature was verified in each of the 8 sympathectomized hands which were studied.

In the hand with vasomotor innervation present, a straight line was generally not obtained when the blood flow was plotted against the temperature by the Arrhenius equation. The circulation in the normal hand is, however, influenced by the temperature of the environment and the needs of the body for the conservation or for the disposal of heat. If the extrinsic factors are balanced against each other in the course of a large number of determinations (see fig. 3), a mean is reached which again can be plotted by the Arrhenius equation, as a straight line.

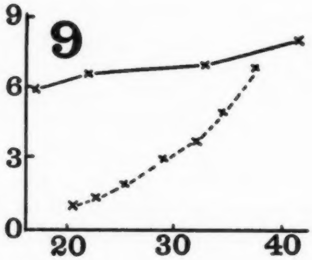
The results of these experiments supported the possibility of a chemical

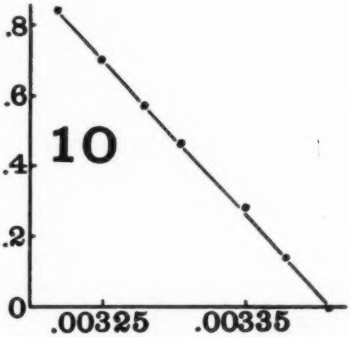
basis for the effect of local temperature on blood flow and suggested the hypothesis that the metabolism of the tissues might be the essential determining mechanism.

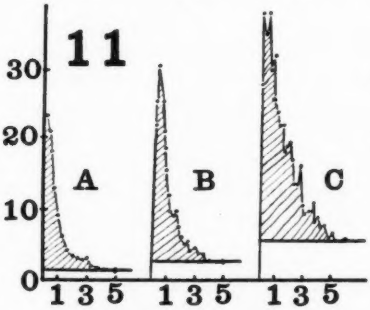
Reactive hyperemia. If the metabolism of the tissues in the hand controlled the rate of circulation, it would follow that the blood flow at any given temperature would exactly fulfill the tissue requirements. Correlatively, if the temperature were maintained at a constant level and the metabolism were thereby stabilized, any obstruction to the flow of blood would be accompanied by certain changes in the tissues which would act as stimulants for an increased circulation. Upon removal of the impediment to the blood flow, the circulation would be increased until the equilibrium in the tissues were again restored. In fact, it has been clearly recognized since the fundamental researches of Roy and Brown (26) that such an increase in blood flow does follow the release of a vascular occlusion. This phenomenon, called reactive hyperemia, has been found to be affected by the local temperature and by the duration of occlusion (18, 21). Lewis and Grant (18) have referred to it in terms of the repayment of a blood flow debt. No exact relationship between temperature, length of occlusion, and the subsequent increased circulation has been found. Quantitative studies were therefore made of reactive hyperemia in order to investigate further the peripheral control of the circulation.

In order to study this reaction the sympathectomized hand was immersed in water at a low temperature, 22.7°C. The rate of blood flow was determined at frequent intervals until it appeared constant at 1.6 cc. per 100 cc. of hand volume per minute. A pressure of 250 to 300 mm. Hg., sufficient to block the inflow of arterial blood, was suddenly applied to the wrist. At the end of exactly 10 minutes the occlusion was released and the rate of blood flow was rapidly and repeatedly determined until, 5 minutes later, the basal level of flow had been regained. The results were then plotted on a large piece of standard graph paper, and the area above a line which represented the basal blood flow was measured with a planimeter. The rate of the chemical process in the hand at 22.7°C. was considered to be equivalent to 1.6 cc. of blood per 100 cc. of hand per minute, and the duration of the occlusion was 10 minutes. A blood flow debt of 16 cc. minutes was therefore established. As can be seen in figure 11, the repayment of the debt in the period following release of the tourniquet amounted to 19.1 cc. minutes. The discrepancy in this case amounted to an excess flow above the theoretical debt of a little less than 20 per cent.

The temperature of the bath in which the hand was immersed was then raised to 27.8°C., and the basal level of flow was determined. The rate of the chemical process in the hand now was represented by a blood flow of 2.9 cc. per minute. At the end of the 10 minute occlusion, the blood flow







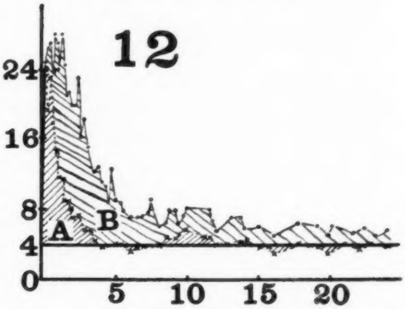


Fig. 9. Effect of changes in local temperature on rate of blood flow in the right hand 6 months after sympathectomy in comparison with the changes observed in the same hand 10 days after operation. Solid line, 10 days after right cervico-dorsal ganglionectomy; interrupted line, same hand 6 months later. Ordinates, blood flow; abscissae, temperature, degrees Centigrade.

Fig. 10. Effect of changes in local temperature on the rate of blood flow in the hand 6 months after sympathectomy when plotted according to the Arrhenius equation. Ordinates, logarithm of the rate of blood flow; abscissae, reciprocal of the absolute temperature.

Fig. 11. Effect of temperature on reactive hyperemia in the sympathectomized hand after 10 minute circulatory arrest: A, 22.7 degrees Centigrade, basal blood flow 1.6 cc. per minute; excess flow after release of tourniquet equivalent to 19.1 cc. minutes; B, 27.8 degrees Centigrade, basal blood flow 2.9 cc. per minute; excess flow equivalent to 29.1 cc. minutes; C, 33.9 degrees Centigrade, basal blood flow 5.7 cc. per minute; excess flow equivalent to 56.7 cc. minutes. Ordinates, blood flow; abscissae, time, minutes.

Fig. 12. Effect of prolonged occlusion of the circulation on the amount of reactive hyperemia. Temperature, 33.8 ± 0.4 degrees Centigrade; basal blood flow 4.0 cc. per minute on both sides. A, right hand after 5 minute circulatory arrest. Excess flow equivalent to 20.3 cc. minutes. B, left hand after 12 minute circulatory arrest. Excess flow, determined simultaneously with that of the right, equivalent to 110.3 cc. minutes. Ordinates, blood flow; abscissae, time, minutes.

was again determined at frequent intervals for 5 minutes until the basal level was reached. As shown in figure 11, the repayment was exactly equal to the theoretical debt. Similarly, at 33.9°C. the debt contracted as the result of a 10 minute occlusion, with the rate of the chemical process now equivalent to 5.7 cc. of blood per minute, was exactly liquidated.

In 17 experiments on 5 different individuals, whether the length of the occlusion was varied from 2 to 12 minutes, or whether the rate of the blood flow was altered by changing the local temperature, the equivalent of the debt was repaid.

This mathematical relationship between blood-flow debt and repayment was only valid within certain very definite limits. It was not exact when the hand was immersed in cold water (see first example, fig. 11). This discrepancy was explained by the probability that the temperature of the tissues of the hand was raised by the inrush of arterial blood at the rate of 23 cc. per minute. The elevation of temperature would naturally increase the metabolic requirements and in this way the basal blood flow level would be elevated. Superimposed upon the blood flow debt created at 22.7°C., there would be an additional demand created by the rise in the temperature of the hand during the first part of the repayment of that debt. The quantitative relationship again failed to obtain when a large debt was created by occlusion of the circulation for a longer period at a higher temperature.

In order to investigate more thoroughly the nature of this apparent deviation from the expected, a patient was slected who had sustained a bilateral cervico-dorsal ganglionectomy one year before. The blood flow was determined simultaneously in both hands at the same temperature. The circulation on both sides was then occluded at the same moment for 2 minutes. After release of the tourniquet the excess blood flow above the basal rate was found to be almost identical in the two hands—9.7 cc. minutes on the left side and 10.1 cc. minutes in the right. The fundamental similarity of reaction of the two hands was thereby proved. The temperature was then raised on both sides to 33.8°C. After a stable level of flow had been attained in each hand, the circulation was occluded on the left side. Tingling commenced at the 3rd minute and distinct pain was felt at the 7th minute. Thereupon the tourniquet was also applied to the right wrist. Five minutes later severe pain was felt in the left hand, which had by then suffered a 12-minute circulatory occlusion. Both tourniquets were then released at the same time. To recapitulate—the blood flow was occluded to the right hand for 5 minutes; tingling was present in the fingers, but there was no pain. On the left side the circulation was occluded for 12 minutes, and severe pain was experienced. The blood flow debt on the right side amounted to 4.0 cc. × 5 minutes or 20.0 cc. minutes. The repayment period lasted for 5 minutes, as shown in figure

12, and the volume flow above the basal requirement amounted to 20.3 cc. minutes, an error of less than 2 per cent. The debt on the left side incurred by a 12-minute occlusion at the same rate of flow amounted to 48.0 cc. minutes. Twenty-four minutes later the blood flow had not yet returned to the basal level. During the 21 minutes, as seen in figure 12, the excess flow above the basal circulation amounted to 110.3 cc. minutes. Whereas on the right side the error was less than 2 per cent after a 5 minute occlusion, on the left side with a 12-minute occlusion the error above the expected repayment was more than 132 per cent. It is clear, then, that the mathematical relationship between debt and repayment is only precise within certain limits. An abrupt deviation from the quantitative relationship between length of circulatory arrest, temperature, and the volume flow after release took place when the asphyxia was marked. It is noteworthy that pain was experienced when this discrepancy was observed.

Discussion. The limitations of the exact quantitative relationship which have been found in the experiments on reactive hyperemia (detailed above) may serve to throw some light on the question of the ultimate cause of the dilatation. A break was demonstrated in the gradation of the reaction which took place after circulatory arrest (see fig. 12). Up to a certain point a linear relationship obtained between metabolic debt and circulatory repayment. A physiological or chemical stimulus might then be considered as exciting the compensatory dilatation. Beyond that point there was a wide divergence in the reaction. The compensatory blood flow greatly exceeded the theoretical value of the debt. The possibility must be considered that a pathological stimulus was initiated when the asphyxia of the tissues became extreme.

It is to be recalled that pain was felt when the debt became excessive. This fact may be related to the pain observed by Moore, Moore, and Singleton (22), when solutions having a hydrogen-ion concentration below pH 6.0 were injected into arteries. With the realization that a tissue pH of 6.4 can be produced by simple functional ischemia (25), it is not hard to believe that an even greater acidity might be produced by prolonged complete arterial occlusion. A moderate concentration of acid formed as the by-product of metabolism in the absence of an adequate supply of oxygen, might serve as the physiological stimulus for vasodilatation. With greater concentration of acid a pathological stimulus might be added. The resultant increase in the circulation would then be the outcome of two different stimuli. Further support for the idea of an added pathological stimulus is supplied by the fact that pain is observed, and that easily recognizable pathological processes such as whealing and blister formation result from prolonged circulatory deficit.

The chief argument which Lewis (16) advances for his belief that the

"H-substance" is responsible for *all* vasodilatation is his contention that there is a complete gradation from the slight reddening caused by moderate warmth to the full "triple response" of tissue trauma. In these experiments a break has been demonstrated in the continuity of the response to circulatory occlusion. It is possible that a pathological stimulus, such as, for example, the accumulation of acid metabolites in too high a concentration, may stimulate additional dilatation through an axon-reflex (2) or through the elaboration of an "H-substance." It is not necessary to ascribe the increase in blood flow after circulatory arrest over the physiological range to any stimulus other than the acid metabolites of the tissues.

These experiments on reactive hyperemia support the original concept proposed to explain the effect of temperature on the rate of circulation in the sympathectomized hand: namely, that the rate of blood flow was determined by the metabolic requirements of the tissues. The phenomenon of reactive hyperemia also makes unnecessary certain of the other hypotheses which were tentatively advanced to account for the influence of temperature on blood flow. Since the increased flow of reactive hyperemia occurred at constant temperature, heat could not have functioned through decreasing blood viscosity (7) or through increasing the calibre of the vessels (15). Furthermore, the presence of the typical response of reactive hyperemia even after degeneration of all the nerves to the extremity (18) would make impossible the participation of posterior root (3) dilators or axon-reflexes (2) in the changes in circulation which accompany alterations in local temperature.

Summary. A method is described for the determination of the blood flow in the hand at constant temperature.

As the local temperature was increased with the subject under basal fasting conditions, the rate of blood flow was increased according to a curve which was characteristic for the individual (fig. 3).

Application of cold to the body caused a decrease in the blood flow both in the normal and in the sympathectomized hand, even though the local temperature was kept constant. Reasons are given for considering that the decrease in the circulation to the sympathectomized hand was the result of adrenal secretion (fig. 5).

Heating the body by means of a cabinet but with the hands immersed in water at a constant temperature produced vasodilatation on the normal side with no change in the circulation to the sympathectomized hand (fig. 6).

With both hands immersed in cold water (17.5 ± 2°C.) novocaine block of the sympathetic nerves to one hand resulted in an increase in the circulation on the injected side (fig. 7).

The circulation was stable in the hand 10 days after sympathectomy

and varied only slightly with changes in the local temperature. Six months later, although there was no evidence of sympathetic nerve regeneration, the blood flow in the hand varied directly with the local temperature (figs. 8 and 9).

Evidence is presented that the changes in blood flow were not the result of adrenal secretion.

The logarithm of the rate of blood flow in the sympathectomized hand is a linear function of the reciprocal of the absolute temperature of the water in which the hand is immersed (Arrhenius equation) (fig. 10). This relationship suggests that the circulation to the sympathectomized hand is controlled by the metabolism of the tissues and that the increase in the blood flow when the hand is warmed results from an acceleration of the metabolic processes.

With the sympathectomized hand in water at different temperatures, the circulation was occluded for ten minute periods. The blood flow was determined at frequent intervals after release of the tourniquet. The amount of reactive hyperemia was found to be equal to the product of the original blood flow at each temperature and the duration of occlusion (fig. 11).

The mathematical relationship between blood flow debt and repayment did not obtain for prolonged occlusion. The reactive hyperemia then exceeded the theoretical requirement (fig. 12). It is suggested that the stimulus which is physiological within certain limits, then becomes pathological and that certain other vasodilator reactions from tissue trauma are produced.

CONCLUSION

The blood flow to the hand is modified reflexly in accordance with the needs of the body as a whole for the dispersion or for the conservation of heat. The circulation in this respect functions as part of the heat-regulating mechanism of the body.

After sympathectomy, the flow of blood is no longer modified in a thermo-regulatory fashion—although adrenine may still exert some influence. The circulation in the sympathectomized hand is then dependent upon the metabolic requirements of the tissues. Under certain circumstances, additional physiological or pathological stimuli influence the rate of blood flow.

The hypothesis first suggested by Roy and Brown is supported: namely, that the local control of the blood flow is mediated through the concentration of metabolites in the tissues. The possibility that the circulatory stimulus is exerted through the acidity of the metabolic products is discussed.

It is a pleasure to express my gratitude to Dr. Edward D. Churchill for his helpful criticisms and suggestions.

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CONTROL OF THE CORONARY BLOOD FLOW BY REFLEXES ARISING IN WIDELY DISTRIBUTED REGIONS OF THE BODY[1]

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In an earlier paper we have reviewed and extended the evidence establishing the dual nature of the efferent coronary vaso-motor apparatus (1). In the present study, data are presented to show that coördinations of the coronary reactions in the normal animal are carried out by reflexes aroused by afferent stimuli arising in widely distributed regions of the body.

The type areas chosen for the tests have been the following: First, afferent sciatic pathways as representative of the great somatic areas, i.e., body, trunk, and limb sources of sensory stimulations leading to coronary reflexes. Second, abdominal and visceral afferent sources, via pathways through the celiac plexus and splanchnic nerves. Third, afferent phrenic stimulations as tests of mediastinal and diaphragmatic sensory areas. Fourth, the afferent neurones of the vagus—searching for special reflex sensory neurones from the cardiac and chest regions.

Reactions of the coronaries to afferent somatic stimulation. The sciatic was chosen as a composite somatic nerve rich in sensory axones from cutaneous, muscular, and skeletal sources. Afferent stimulation of the sciatic, even of the mildest intensity, produces profound reflex acceleration of respiratory movements with great augmentation of respiratory volume, also pronounced systemic vaso-motor and cardiac readjustments. The systemic reflex vaso-motor effects from sciatic stimulation were analyzed by Bradford and Dean (2) and by Reid Hunt (3).

The evidence offered in this report was obtained from numerous tests by stimulating the central end of the sciatic while recording the rate of outflow of the coronary sinus. The variation in rate of flow from the coronary sinus was accepted as a measure of the variations in volume of the coronary arteries contributing to the sinus pathway, therefore, typical of general coronary reactions. Changes in the coronary flow have been modified passively in some tests by the coincident uncompensated changes

[1] Appreciation and grateful acknowledgment are here extended to the National Research Council for two grants for defraying the expenses of the experiments on the reflex pathways in the control of the coronary blood vessels.

in blood pressure, or by other uncontrolled experimental variations difficult to eliminate completely. However, the tests have been outstanding and convincingly positive.

Fig. 1. Reflex dilatation of the coronaries in response to mild afferent stimulation of the central end of the sciatic. The rate of flow from the coronary sinus is indicated by the pitch of the oblique tracing of the float of the volume manometer. The standard volume of the manometer is 0.71 cc. per millimeter of length. Blood pressure recorded by mercury manometer. Time in 5 second intervals. Reduced 50 per cent.

Protocol 1, presenting data from figure 1

Animal 11—14: Stimulating the central end of the right sciatic, 9 cm., coronary efferent nerves intact. Blood pressure equalized. Note that there is a diphasic reaction, of 28 per cent increase in coronary flow at 30 seconds and a decrease to normal at 90 seconds. The decrease occurs in the presence of a rise of blood pressure that operates to produce a mild passive increase in coronary flow.

TIME	BLOOD PRESSURE	CHANGE	HEART RATE	CHANGE	CORONARY FLOW	CHANGE
		per cent		*per cent*		*per cent*
10″ before	40.0		141		87	
10″ after	43.6	9	144	2	100	15
30″ after	46.8	17	144	2	111	28
60″ after	44.0	10	144	2	90	3
90″ after	46.1	15	144	2	87	0
125″ after	47.7	19	144	2	88	1

We offer in illustration a single typical experimental figure with detailed tabulations of its data. This is supported by a brief statistical table.

In animal 13—8, figure 2, table 1, the immediate effect on the coronary flow was a slight passive fall in rate of flow shown at the moment of lowest blood pressure, and within the latent period of the increase in active coronary reflex response. At 20, 60 and 150 seconds the coronary flow had increased in an active and persistent reflex coronary dilatation. In this test the heart rate does not influence the degree of coronary dilatation. The heart rate was considerably slower in the first 45 seconds, during the time the coronary flow was augmented, but returned to the initial rate after 120 seconds. The blood pressure was equalized to approximately 75 mm. of mercury, and showed only slight acute variations. An acute fall of arterial pressure of -9 per cent occurred within 7 seconds of the beginning of stimulation. The variations of blood pressure are in the negative direction and passively produced the decrease in coronary flow at that moment.

In animal 19—12, with 12 cm. coil, sciatic stimulation produced a progressive fall in arterial blood pressure, amounting to -17 per cent after 110 seconds while the heart rate remained constant. The variable coronary flow showed a maximal augmentation of 13 per cent at 85 seconds. The lower arterial pressure admittedly lowers the coronary flow. The positive coronary reflex dilatation of 13 per cent should be greater by the amount of the passive neutralization. The weak stimulus used to produce this very effective reflex dilatation is an index of the very sensitive vascular autonomic apparatus often observed in dogs.

In animal 17—19, the left sciatic yielded pronounced coronary dilatation. The heart rate remained constant, hence the fall in blood pressure was due to systemic vascular changes. The simultaneous reflex coronary dilatation increased the flow from a normal of 90 to 117 cc. in 60 seconds. This is an increase of 30 per cent, or 50 per cent if calculated on the basis of the normal coronary flow of an earlier test in this animal.

The numerous sciatic tests yielded great variation between pure dilatation of the coronaries at one extreme and pure constriction at the other. It is clear that the coronary reflex is a double one in the sense that simultaneous coronary dilator and coronary constrictor impulses are impinging on the coronary vessels. The net reaction is the sum of these impulses in the sense used by Hunt (3) in comparing the tonic bineural control of the heart rate. When the afferent stimulus is weak, the reaction, as a rule, is one of reflex coronary dilatation. If the stimulus is strong the constrictor reflex is, or at least may be, preponderant. In such a composite there is no experimental method whereby the proportionate contribution of the two factors in the compound result may be specifically evaluated.

Sciatic stimulation after atropine. Atropine has little influence upon the vaso-motor coronary reflex. In ten sciatic tests after atropine, with

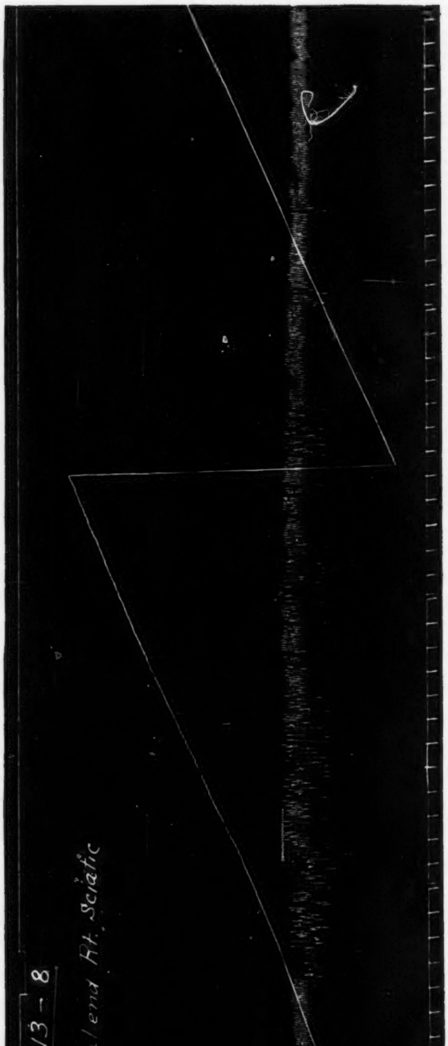

Fig. 2. Reflex coronary dilatation associated with violent respiratory augmentation and irregularity of cardiac rate on stimulating the central end of the sciatic, 10 cm. Time in 5 seconds. Reduced 50 per cent.

both vagal and sympathetic efferent paths intact, a frank coronary constriction occurred in only one, initial coronary dilatation followed by constriction was obtained in five, initial constriction followed by dilatation in one, and uncomplicated coronary dilatation in three tests.

Cutaneous-sensory and musculo-sensory reflexes. Cutting the skin during an operation produced reflex coronary dilatation. Cutting the sciatic nerve itself produced a reflex coronary constriction.

Nine tests on two animals were made in an effort to demonstrate coronary reflexes from stimulation of musculo-sensory nerves. The motor branches of the sciatic to the vastus externus muscle were stimulated. With all cardiac nerves intact, stimulation of the central end of this motor nerve in animal 13—9, at 10 cm., produced a coronary dilatation of 11 per cent. The blood pressure was only 4, 2, and 1 per cent above the initial

Protocol 2, presenting data from figure 2

Animal 13—8: Stimulating central end of sciatic, 10 cm. Coronary dilatation, violent respiratory movement and prolonged irregularity of heart rate. Heparin, but no atropine. All nerves intact.

TIME	BLOOD PRESSURE	CHANGE	HEART RATE	CHANGE	CORONARY FLOW	CHANGE
		per cent		*per cent*		*per cent*
10″ before	76		114		33	
7″ after	69	−9	108	−5	30	−9
10″ after	71	−7	108	−5	33	0
20″ after	71	−7	102	−11	37	12
45″ after	71	−7	90	−21	40	21
60″ after	78	3	108	−5	40	21
90″ after	72	−5	96	−14	37	12
120″ after	76	0	114	0	39	18
150″ after	75	−1	126	11	39	18

pressure. When the stimulus was strong, 4 cm., there was a mild reflex fall of blood pressure and a decrease in coronary flow. In animal 14—12, 6 cm., at 55 seconds, there was a positive coronary dilatation of 15 per cent associated with a fall of −6 per cent in blood pressure. In test 14—13, at 20, 30 and 35 seconds after stimulation, the coronary flow increased to 8, 2, and 3 per cent respectively. The blood pressure decreased slightly. The data all point to the presence of musculo-sensory reflex coronary dilatations, though more extensive evidence is desirable.

Coronary reflexes from abdominal visceral sources. We have applied the method of the coronary sinus to the study of the variations of coronary flow in response to afferent stimulation of different regions in the abdominal viscera.

Variations in coronary blood flow occurred in response to stimulation

of all types of afferent abdominal nerves. The most general and complex of the abdominal pathways is through the celiac ganglion and splanchnic nerves. Stimulation of the celiac ganglion produces reflex dilatation of

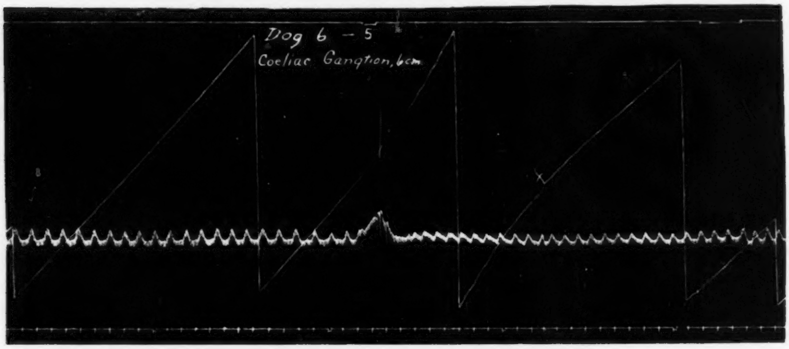

Fig. 3. Reflex coronary dilatation of 42 per cent upon stimulating the celiac ganglion, 6 cm., all nerves intact, no atropine. Time in 5 seconds. The coronaries were in equilibrium following a preceding reflex dilatation.

The coronary constriction in the after-period is interpreted as due to a simultaneous constrictor reflex which was over-balanced earlier in the test by the more powerful dilator reflex. Coronary dilatation occurred in each of 6 successive celiac stimulations in this dog. At *f* the recording paper stopped for a moment. Reduced 57 per cent.

Protocol 3, presenting data from figure 3

Animal 6—5: Stimulating the celiac ganglion, 6 cm. Heparin, no atropine. Blood pressure equalized.

TIME	BLOOD PRESSURE	CHANGE	HEART RATE	CHANGE	CORONARY FLOW	CHANGE
		per cent		*per cent*		*per cent*
10″ before	75		158		84	
10″ after	74	−1	158	0	105	25
20″ after	77	3	173	10	119	42
30″ after	75	0	170	8	119	42
40″ after	74	−1	170	8	112	33
60″ after	73	−3	163	3	84	0
75″ after	74	−2	163	3	67	−20
90″ after	74	−2	156	−1	60	−29
105″ after	74	−2	157	−1	60	−29

the coronary vessels. This may or may not be followed by coronary constriction. The coronary dilatation may be unexpectedly large. This is shown in animal 1—18, 4 cm.coil position, in which the maximum dila-

tation amounted to an increase of 180 per cent over the initial normal. This extreme dilatation was due in part to rise in blood pressure.

The celiac ganglion is the way-station of a large proportion of the afferent visceral neurones. The production of reflex coronary responses upon stimulation of the celiac ganglion is adequate proof of the presence of viscero-coronary reflex arcs.

The change in volume of coronary flow in response to celiac stimulation is strikingly like that obtained from the sciatic. When the stimulus is very mild, reflex coronary dilatation is the primary picture. When the intensity of the stimulus is increased coronary dilatation occurs at first, often of greater volume than the response to a milder stimulus. The initial dilatation may be succeeded by constriction expressed in a diphasic record.

This is illustrated in animal 6—5, figure 3, and protocol. The coronary reflex produced at first a sharp increase in coronary flow, 42 per cent within 20 seconds. The dilatation was followed by a late coronary constriction which at 105 seconds amounted to -29 per cent of the initial volume of flow and was still -16 per cent after 140 seconds. This test illustrates one of the most extreme diphasic reactions obtained. Table 1 presents further data from a variety of tests, some simple reflex dilatations, others pure coronary constriction.

We draw the conclusion that definite reflex central paths exist as between the abdominal visceral sensory neurones and the efferent neurones to the coronary blood vessels. These paths are represented by both dilator and constrictor arcs. The dilator arcs are very sensitive to weaker stimulations, but dilatations are often overbalanced by powerful and dominant coronary constrictions.

Electrodes were applied to certain contributory nerves to the celiac ganglionic complex, especially those arising in the region of the gall bladder and common cystic duct. The coronaries respond to stimulation of this region by dilatation and an increase of blood flow.

In view of the part played by visceral cramps and similar abdominal conditions in the development of other vascular reflexes, we explored a number of abdominal branches of the vagus nerve terminating in different regions of the gastric wall and in hepatic structures. The evidence of coronary reflex control through afferent vagal pathways has, in our hands, been slight, considered in terms of volume of change. Nevertheless, the reaction to afferent vagal stimulation is more often a positive coronary dilatation.

Penetrating the diaphragm of the dog there are three principal divisions of the vagus plexus derived from recombining divisions of the right and left vagus trunks in the lower thorax.

Each of these divisions contains afferent fibers as well as efferent sym-

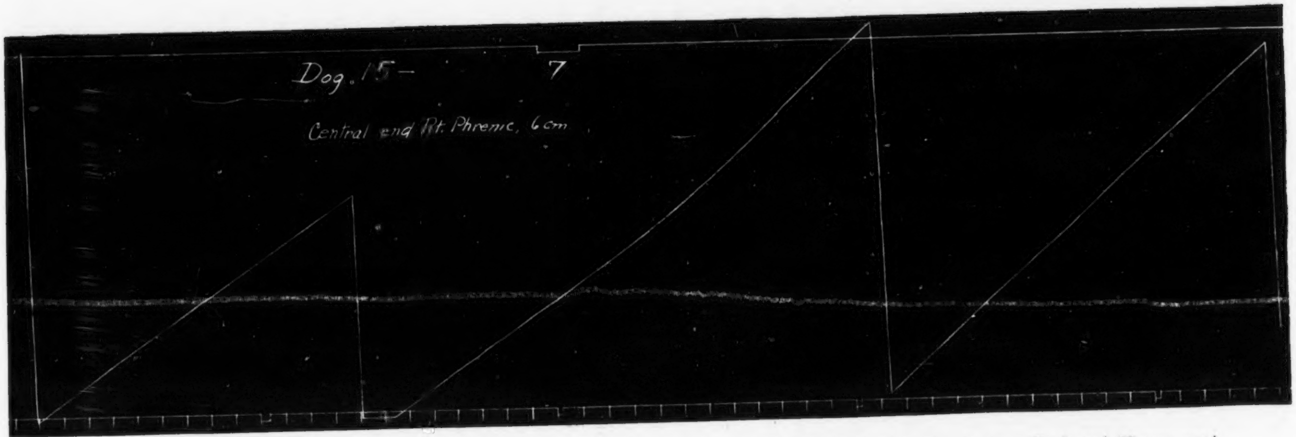

Fig. 4. Dilatation of the coronary vessels upon stimulating the central end of the right phrenic, 6 cm. Reduced 50 per cent.

pathetic components. Hinrichsen and Ivy (4) have published data and figures illustrating coronary reflex dilatations in response to stimulations of the central end of gastric branches of the vagus (vago-sympathetics). Their experiments support the view on which we have data that afferent pathways from gastric regions are reflexly connected with the coronary efferent nerves through reflex centers.

Afferent neurones in the phrenics induce coronary reflexes. Upon sectioning the phrenics or after stimulating their central ends we have in many experiments observed changes in blood pressure, heart rate, and coronary flow which strongly suggest an intimate reflex relationship between afferent phrenic neurones and the efferent neurones to the heart and coronary blood vessels.

Individual phrenic experiments have varied greatly and have often been quite negative. In positive tests the coronary flow and heart rate have

Protocol 4, presenting data from figure 4

Animal 15—7: Stimulating central end right phrenic, both phrenics cut; 6 cm. Heparin, atropine. Arterial pressure equalized.

TIME	BLOOD PRESSURE	CHANGE	HEART RATE	CHANGE	CORONARY FLOW	CHANGE
		per cent		*per cent*		*per cent*
10″ before	68		160		61	
15″ after	68	0	160	0	65	7
30″ after	66	−3	167	4	73	20
45″ after	62	−9	169	5	83.5	37
60″ after	58.4	−14	169	5	82	34
75″ after	56	−18	169	5	82	34
90″ after	54.7	−20	169	5	79	30

both been augmented upon sectioning the phrenics. Stimulation of the central end of a phrenic in certain instances has been followed by pronounced reflex coronary dilatation, animal 15—7.

Coronary reflexes aroused by stimulation of afferent fibers in the vagus. It was through the vagus that Anrep and Segall (5) produced the first demonstration of coronary reflex control. This experiment we have confirmed. We find, however, that the vagus reflex may produce either coronary dilatation or coronary constriction.

The coronary reflex responses to purely afferent cervical vagal impulses is illustrated by the 12 tests in table 4. The coronary responses are of two types; dilatation in some tests, constriction in others. In animal 6, test 34, there was a large dilatation of 38 per cent in the first 30 seconds, on a second test, 6—35, the dilatation was reduced −7 per cent. Other vagal stimulations yielded purely reflex coronary dilatations. Still others

TABLE 1

Stimulation of the central end of the sciatic nerve

ANIMAL AND TEST NO.	STRENGTH OF STIM. IN COIL POSITION	TIME FROM BEGINNING OF STIM.	PER CENT OF CHANGE			REMARKS
			Blood pressure	Heart rate	Volume coronary flow	
	cm.	*sec.*				
3—15	6	15	21	4	42	Atropine
		60	8	8	21	Equalizer not used
10—11	10	7	17	3	12	No atropine
		12	−7	0	−13	
		35	−9	3	−8	
		80	6	0	−14	
10—18	10	30	−4	0	17	Atropine
		75	−10	0	8	
11— 9	10	7	9	0	8	No atropine
		20	7	0	6	
		35	3	0	−2	
		90	6	0	−13	
11—17	9	10	0	0	4	Atropine
		30	5	0	14	
		45	1	0	7	
13—13	10	10	−8	0	−4	No atropine
		20	3	0	−5	
		75	1	1	−9	
		105	−2	1	−12	
13—16	6	20	1	0	4	No atropine
		50	4	0	10	Deeper anesthesia than tests 8–10
14—10	10	12	8	3	1	Phrenic nerves cut
		25	−9	0	−11	No atropine
		40	−9	−3	−13	
14—14	8	13	10	11	5	Phrenics cut
		30	−6	8	−18	No atropine
		90	−1	11	−15	
17—19	12	20	−13	0	9	Atropine
		60	−7	0	30	(+50 if calculated by the normal
		90	−9	6	30	of test 18)
17—21	12	25	−2	0	−3	(+55 if calculated by the normal
		90	−1	0	−9	of test 18)
						Change in sign of reflex reaction after repeated stimulation
18— 9	13	15	18	−4	10	No atropine
		35	−9	0	7	
		80	−12	0	19	
19—12	12	8	5	0	17	No atropine
		50	−6	−2	−7	
		85	−11	−2	13	
		110	−17	−2	7	
19—23	12	10	−2	0	6	Phrenics cut
		30	−18	0	1	No atropine
		105	−3	4	27	

gave only coronary constriction; in animal 14—40, the reflex coronary constriction from the right vagus amounted to −30 per cent. However, the amount of coronary change is sometimes quite slight, not so much an expression of a low reaction as of the neutralizing effects of simultaneous dilator and constrictor reflexes. The tests of table 4 were all made after bilateral vago-section. Four of the tests induced coronary dilatation and eight constriction. Since the vagi are cut the coronary constrictor nerves cannot take part in the reflex. The explanation that seems to apply is that of a reflex inhibition of the coronary dilator tone. Coronary constriction, as an effect of stimulating sensory neurones of the vagus, has not previously been reported.

TABLE 2

Stimulation of central end of motor nerve to vastus externus

Atropine preceded test 13-26

ANIMAL AND TEST NO.	STRENGTH OF STIM. IN COIL POSITION	TIME FROM BEGINNING OF STIM.	PER CENT OF CHANGE			REMARKS
			Blood pressure	Heart rate	Volume coronary flow	
	cm.	*sec.*				
13— 9	10	20	4	0	11	No atropine
		70	2	0	8	
		110	1	0	10	
13—10	8	39	2	0	5	No atropine
		77	1	0	1	
13—26	4	37	−1	0	−2	Atropine
		72	−6	0	−8	
14—11	10	25	−1	0	−6	No atropine
14—12	6	18	−4	3	0	No atropine
		55	−6	3	15	
14—13	6	20	−1	0	8	No atropine
		30	−5	0	2	
		35	−4	0	3	

The vagus was stimulated in the mid-cervical region, hence below the sensory nerves of the carotid sinus. The possibility of coronary reflexes from the carotid sinus is yet to be determined. This statement also applied to the theoretically more direct sensory paths from the heart itself. This field has, at present, no experimental data.

Many investigators have been interested in the distribution of sensory nerves to the heart itself and its immediate adjacent structures. Sutton and Lueth (6) demonstrated experimentally that the surface of the heart of dogs is supplied by pain inducing nerves. Mechanical stimulation was applied to the coronary arteries in prepared animals that were otherwise normal. The responses to carefully executed tests indicated delicate sensory reactions.

White, Garey and Atkins (7) have recently published experimental and clinical evidence that cardiac sensory neurones reach the thoracic cord via the posterior roots of the first five dorsal spinal nerves. They used the method of Sutton and Lueth to produce cardiac ischemia and pain. In

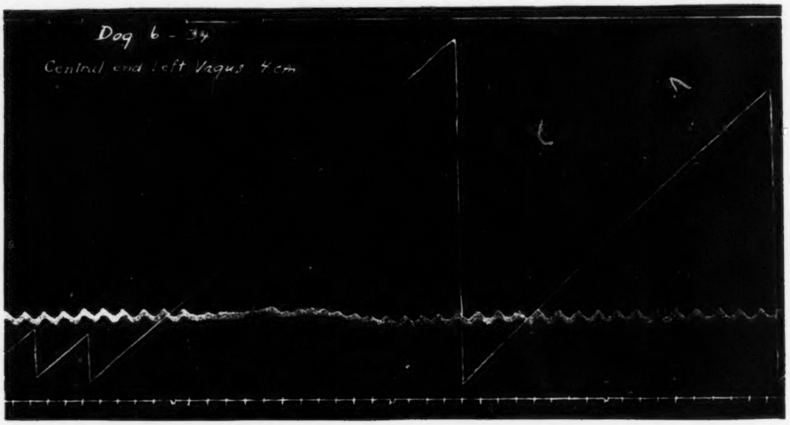

Fig. 5. Coronary dilatation in response to stimulation of the central end of the left vagus, 4 cm. Time in 5 seconds. Reduced 60 per cent.

Protocol 5, presenting data from figure 5

Animal 6—34: Stimulating the central end of left vagosympathetic, 4 cm. Blood pressure equalized. Ether, morphine, atropine, heparin.

TIME	BLOOD PRESSURE	CHANGE	HEART RATE	CHANGE	CORONARY FLOW	CHANGE
		per cent		*per cent*		*per cent*
10″ before	57		151		60	
10″ after	57	0	151	0	60	0
20″ after	60	4	151	0	76	27
30″ after	59	4	165	10	83	38
45″ after	54	−5	158	5	73	22
60″ after	52	−10	155	3	64	7
90″ after	55	−4	151	0	70	17
120″ after	55	−4	150	−1	67	12
150″ after	55	−4	150	−1	62	3

a series of dogs they showed that evidence of pain persisted after double vagotomy, after removing the stellate ganglia, was reduced on sectioning the posterior upper dorsal roots on one side only and disappeared completely after cutting the first five dorsal roots on both sides.

Twenty-three paravertebral injections of alcohol in clinic patients with anginal pain in the Massachusetts General Hospital gave good results in 47.8 per cent, fair in 30.4 per cent, failures in 13.1 per cent of the cases and no deaths.

Ionesco and Enachesco (8) demonstrated that a branch of the 6th thoracic sympathetic ganglion, which travels along the azygos vein to the

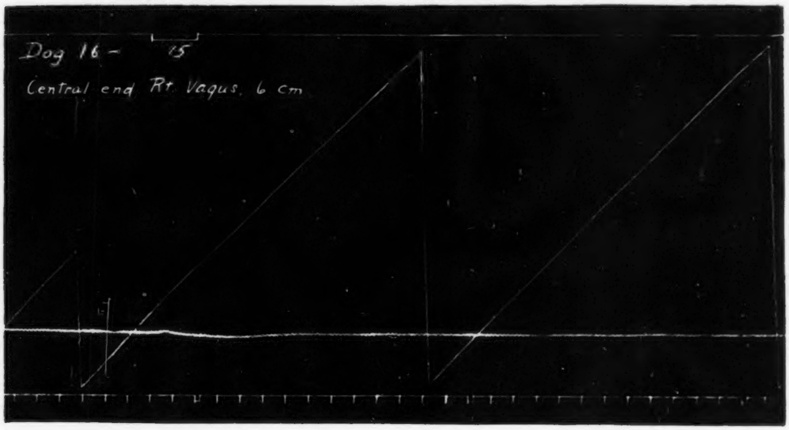

Fig. 6. Constrictor reflex from central stimulation of the right vagus, 6 cm. Time in 5 seconds. Reduced 55 per cent.

Protocol 6, presenting data from figure 6

Animal 16—15: Stimulating the central end of the right vagus, 6 cm. Blood pressure equalized. Heparin. Both vagi cut.

TIME	BLOOD PRESSURE	CHANGE	HEART RATE	CHANGE	CORONARY FLOW	CHANGE
		per cent		*per cent*		*per cent*
10" before	42		204		96	
15" after	38	−10	198	−3	84	−12
30" after	40	−5	192	−6	84	−12
45" after	40	−5	192	−6	84	−12
70" after	40	−5	192	−6	90	−6
90" after	40	−5	192	−6	88	−8
120" after	40	−5	192	−6	85	−11

heart, contains afferent fibers. The evidence is in the fact that stimulation of the central end of this nerve produces reflex acceleration of the heart rate.

In contrast to these demonstrations, which point to definite cardiac sensory neurones, Capps (9) explored various areas of the diaphragm, the

mediastinum, the inner surface of the pericardium, and the cardiac wall itself. He used a mechanical probe as a stimulating agent and applied

TABLE 3

Celiac stimulations

ANIMAL AND TEST NUMBER	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF CHANGE			REMARKS
			Blood pressure	Heart rate	Volume coronary flow	
	cm.	*sec.*				
1—33	6	15	29	0	15	Vagi cut
		25	48	0	39	Phrenics cut
		35	39	0	55	Accelerators intact
		50	23	0	31	No atropine
		90	15	0	7	
2—12	8	15	12	0	16	Vagi intact
		30	8	0	9	No atropine
		45	5	0	9	
		60	−2	0	−8	
		90	−5	0	−15	
2—13	6	20	28	0	25	Vagi intact
		60	8	0	2	No atropine
		90	0	0	−17	
4— 7	6	10	4	6	13	Vagi intact
		30	−2	3	−3	Atropine
		60	−7	0	−13	
		100	−7	0	−12	
5— 2	8	15	−1	0	0	Vagi intact
		30	0	0	−4	Atropine
		60	−1	0	−9	
		90	−1	0	−10	
6— 9	9	15	1	0	10	Vagi intact
		30	−1	0	18	No atropine
		60	−1	0	14	
7—11	6	20	13	4	14	Vagi intact
		40	3	4	−2	No atropine
		60	−3	0	0	
8— 8	10	15	0	0	0	Vagi intact
		30	−1	0	7	Atropine
		50	−1	0	14	
		80	−1	0	5	
9— 7	10	25	−10	3	−16	Respirations weak
		65	−6	−3	−10	No atropine
9—18	8	30	17	0	33	Vagi cut
		80	24	0	57	Atropine

tests to living, conscious humans. Patients experienced no pain from mechanical contact of the tip of the probe moved over the inner wall of the pericardium or of the surface of the heart.

An extra systole, mechanically stimulated, was readily perceived in consciousness, but pain was not induced. The evidence indicates that patients have a very low contact sensitivity of the pericardium and walls of the heart itself. On the other hand, definite sensory responses and pain are aroused by the probe applied to the thoracic and abdominal or visceral surfaces of the diaphragm and the adjacent portions of the mediastinal wall. It is regrettable that coronary measurements cannot be performed on man.

TABLE 4

Stimulations of the central end of the vagus. Both vagi cut

After atropine

ANIMAL AND TEST NUMBER	SIDE CERVICAL VAGUS NERVE	STRENGTH OF STIMULATION IN COIL POSITION	TIME FROM BEGINNING OF STIMULATION	PER CENT OF CHANGE		
				Blood pressure	Heart rate	Volume coronary flow
		cm.	*sec.*			
6—34	Left	4	30	4	10	38
			60	−10	3	7
6—35	Right	4	50	−1	4	−7
6—41	Left	4	40	−1	−4	−6
			125	−7	−4	−16
7—29	Left	6	15	−9	8	−7
			65	−6	−4	−17
9—28	Right	8	45	−3	−9	16
12—27	Right	6	50	−1	0	18
14—40	Right	6	35	−2	0	−30
16—15	Right	6	30	−5	−6	−12
18—45	Right	6	10	13	−5	−1
			40	2	−5	−13
19—35	Right	10	20	−5	3	8
			60	−2	3	7
19—40	Left	8	30	−4	0	−11
			65	4	0	1
19—41	Right	10	80	4	3	14

GENERAL DISCUSSION. In review of the entire group of coronary reflex experiments emphasis should be given to the universality with which afferent stimulations bring about reflex coronary responses. All the great representative regions of the body have been tested—the somatic, abdominal, the special regions of the diaphragm and pericardium, thoracic, and cervical vagal afferent mechanisms. These are the different types of afferent nerve channels involved in the greater coördinations of the body as a whole.

A point of emphasis is the obvious ease with which coronary dilatations are reflexly produced. This is the typical response to all the milder tests

whatever the region examined. Reflex coronary dilatations may be associated with extensive vaso-constriction in other regions of the body. Conditions that arouse autonomic responses in general are the initial and milder forms of change in motility, such as movement, digestion, body position, milder changes in intensity of sensory activity expressed in the word excitement.

Profound stimulation, that is over-stimulation, leads to reflex coronary constriction. The reflex constriction may be immediate, or follow a brief initial reflex dilatation. The coronary constriction is independent of the general systemic vascular reactions. Constriction may occur from stimulation of any and all regions of the body via sciatic, celiac, vagal, etc., reflexes.

Coronary pathways may be decidedly modified by sectioning known individual efferent paths and still the organism will have adequate channels for carrying out coördinating coronary reflexes, as will be clear upon considering the widespread and bilateral duplication of efferent coronary neurones. Indeed, very radical denervations must be produced to seriously influence the efficiency of the coronary reflex apparatus, a point of very great clinical significance.

Evidence indicating coronary reflex dilatations in response to sensory stimuli arising during voluntary muscular activity is being assembled in a separate report.

Preliminary experiments, only, have been made in the solution of the question whether sensory nerve impulses may arise in the heart itself, that ultimately may produce reflex changes in coronary flow. These tests are still in progress.

We have not paid particular attention to the special sense organs as sources of coronary reflex stimulation, but confidently believe such stimuli would induce the usual type of bineural coronary reactions.

SUMMARY

1. The volume of the coronary flow is influenced by active vaso-motor reflexes induced by afferent stimulations arising in all parts of the soma and viscera of the body.

2. The above deduction is based on numerous tests applied to the central or afferent ends of the sciatic, the splanchnic, the phrenic, and the vagus nerves.

3. The coronary reflex reactions are both dilator and constrictor in type.

4. The coronary dilatations are more readily obtained, are greater in volume, and occur in response to stimuli of the milder and more normal ranges of intensity.

5. The coronary reflex is often diphasic in type in that the initial reflex dilatation may be followed by a late coronary constriction.

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ERRORS OF ROUTINE ANALYSIS IN THE COUNTING OF LEUCOCYTES[1]

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Generally stated, two explanations have been advanced to account for the wide range of physiological oscillations in the leucocyte counts reported in the literature. According to one view, the major variations are not physiological but are inherent in the technical procedure, although it is admitted that after the utmost refinement of technique there still may remain certain "residual fluctuations" which can represent either physiological variations or variations due to factors interjected in the process of sampling (Ponder, Saslow and Schweizer, 1931). The alternative view is expressed by Shaw (1926): "With modern methods of counting the amplitude of physiological oscillations is as a rule too great to be attributed to faulty technique"; these he states may in part be ascribed to peripheral vasomotor variations and when controlled the remaining fluctuations probably represent physiological oscillations in the systemic blood.

It is impossible to decide between these points of view or to determine their common basis of truth until we know what constitutes a reliable blood count. What is still more important is the fact that hitherto there has been a complete neglect to adopt a *constant mathematical* or *statistical* measure for the purpose of evaluating the errors and determining their probable significance. There is also a wide disagreement in the limits of accuracy obtainable with available methods. An analysis of the errors therefore has seemed imperative and is presented in this communication as a basis for a subsequent evaluation of physiological variations.

Analysis of method. This analysis has been made to determine a practical degree of accuracy of total leucocyte counting when the commonly employed methods and appliances are used. The appliances which we have investigated include sampling and diluting pipettes of the Thoma-Zeiss, Trenner automatic and Haak automatic designs, and counting chambers of both the Levy and Levy-Hausser designs with the improved

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Neubauer ruling. In all of our analyses we have used dilutions of the blood of 1/20 and unit areas of one square millimeter on the hemacytometer (unit volume = 0.1 cu. mm.); a constant number of unit areas has been counted for each determination of a total leucocyte count. Türk's fluid has been used as the diluent.

Sources of error. In any routine analysis each step in the process may introduce some degree of error. The possible sources of variation in the purely technical procedures of blood counting may be listed as follows: 1, filling the sampling pipette; 2, dilution of the sample; 3, variations in calibration of the appliances; 4, personal factor, including that of counting, and 5, "subsampling"—a term we have applied to a group of three more or less dependent variables the results of which cannot be separated in routine analysis and therefore must be considered as a whole; they are: variations due to a, mixing of the cells and diluting fluid; b, filling the counting chamber by capillarity, and c, settling of cells by chance on the ruled field of the counting chamber. It is obvious in such a variety of procedures that we are dealing in the end with a *compound variable* an analysis of which can be made only by a statistical approach.

Our experience, which is amply substantiated in the literature, has shown that variations due to the first four sources mentioned above are quite small in comparison to those introduced by the fifth, or the processes of "subsampling"; that is to say, the apparatus of responsible manufacturers in the hands of experienced workers introduces practically negligible variations and the problem of "subsampling" is the only one of major significance in estimating the technical errors. It is obvious that all other factors in the technical procedure would be submerged in the much larger variations due to "subsampling" and therefore that they cannot be individually evaluated, nevertheless the chance summing of these variables may possibly increase the *range* of the *compound variable.*

The present discussion will be confined to (I) errors of "subsampling," and (II) the *compound variable* or the final error of analysis in the determination of leucocyte counts.

I. Subsampling. *a. Mixing of cells and diluting fluid.* Failure to obtain an even mixture of blood and diluting fluid through inadequate mixing was recognized by the earlier investigators as an important source of error (Potain, 1867; von Limbeck, 1901). The ability to secure an even mixture has been a point of controversy. Thus, Student (1907) states, that—"owing to the difficulty of obtaining a drop representative of the bulk of the liquid the larger errors will probably be due to this cause and it is usual to count several drops—"; on the other hand, Purdy (1925), sees "no obvious reason why this should be so," since with his technique he found "no difference between the evenness of spread of the cells in the bulk dilution as compared with that of the cells in the counting

chamber." "This result," Purdy states, "is in accordance with the findings of a number of workers (gives references), hence it is clear that rather than take several drops, it is distinctly more economical of time and labour to use one drop only, and count of it as large an area as is necessary to give the desired accuracy in the results."

We have recently published results obtained with mechanical devices which employed two different principles of mixing the cells and diluting fluid (Bryan and Garrey, 1934). One of these, a shaker which shakes the diluting pipette in only one plane, failed to give an even mixture thus resulting in surprisingly large variations in the leucocyte counts obtained even from the contents of a single pipette, while the other, a rotor device, gave the most uniform dispersions that we have been able to obtain after trying a variety of procedures. Using the rotor, all drops taken from a given pipette gave consistently uniform counts. We have obtained equally uniform dispersions by the hand method of rotation described by Potain (1867) and by Cabot (1932).

b. Filling the counting-chamber. As pointed out by Osgood and Haskins (1931), considerable error may be introduced by improper technique of filling the counting-chamber but these errors may be avoided with proper precautions. We have confirmed the importance of the precautions which these authors stressed but feel that a further discussion of this source of error would be supererogatory in view of their adequate treatment of the subject.

c. Settling of cells by chance on the ruled field of the counting-chamber. When the factors of mixing the cells and diluting fluid, and filling the counting-chamber are properly controlled, the problem of subsampling resolves itself practically into a determination of the error of random sampling due to the chance distribution of cells on the ruled field of the counting-chamber. Student (1907), in his study "On the Error of Counting with a Haemacytometer" has shown that the distribution of small particles in a liquid theoretically will follow the Poisson Law or "Law of Small Numbers" and that the distribution of cells which have settled out of a liquid on the ruled field of a hemacytometer likewise follows this law, but he has also clearly pointed out that as m, the mean number of particles per unit volume (i.e., "prism standing on unit area"), or per unit area on the hemacytometer, increases, the distribution described by this law approaches the *normal curve.* Thus in determining the distribution of cells on unit areas of a hemacytometer, the frequency would be expected to follow a Poisson series when m (i.e., the number of cells expected on the average on each unit area) is small and to approach the *normal* or Gaussian curve when m is large. In dealing with cell frequencies (i.e., the number of cells per unit area) met with ordinarily in the counting of leucocytes on unit areas of *one square millimeter*, the standard deviations

determined from the Gaussian Law and from the Poisson Law, do not differ very greatly though according to the latter law the frequency distribution to be expected is positively skewed (i.e., a larger per cent of the cases will be in excess than in defect of the mean). This fundamental tendency toward asymmetry has not been found and, in reality, was not to be expected in our final results since, as will be shown, several sources of variation influence our actual distribution of cells on the counting field and, as pointed out by Yule (1932), p. 307, "the forms of the frequency-distribution of the elementary variables affect the final distribution less and less as their number is increased: only if their number is moderate, and the distributions all exhibit a comparatively high degree of asymmetry of uniform sign, will the same sign of asymmetry be sensibly evident in the distribution of the compound variable."

Procedure. In studying the distribution of leucocytes on unit areas of one square millimeter on the hemacytometer it is necessary to make many preparations in order to obtain a sufficient number of unit observations to deal with statistically, since each counting field contains only nine unit areas of one square millimeter each. Consequently, as pointed out above, variations due to the factors of mixing the cells and diluting fluid and of filling the counting-chamber by capillarity cannot be separated from the variations due to the chance distribution of cells as they settle out of solution. In addition, errors of counting contribute to the variability in the recorded number of cells per unit area, although the variations due to this cause are not very great.

Data concerning the distribution of leucocytes on the one square millimeter areas of a counting-chamber were obtained in the following manner: In *each experiment*, as many counts as possible were made from the contents of a single pipette (the number of counts varied with the size and type of pipette used), two drops were wasted between successive counts in order to clear the capillary of the pipette each time; all sources of variation except those inherent in "subsampling" were controlled since each experiment involved only one sample of blood and one pipette, with a single individual making the count, using a single counting-chamber. On the average fifteen counts were made from each pipette. Each count recorded the number of cells on each square millimeter of the nine square millimeter area on one side of the counting-chamber. Thus an average of 135 millimeter-squares were counted from the contents of each pipette; this is a sufficient number to permit accurate determinations of the statistical measures for each experiment (results obtained on small pipettes which gave fewer than ten counts have not been included in our statistical analysis). All of these experiments have employed the use of the mechanical rotor previously described (Bryan and Garrey, 1934) for mixing cells and diluent, and the technique of filling the counting-chamber described

by Osgood and Haskins (1931). We have previously published original data showing the frequency distribution in actual numbers of cells per unit area in a typical experiment of this kind, together with the statistic measures calculated therefrom and a graphical representation of these data (Bryan and Garrey, 1934). We present herewith table 1 which is a tabulation of the statistic measures and a summary of the results obtained in 25 such experiments. It will be observed from column 3 of this table that the standard deviations in the 25 experiments are all of the same order of magnitude and do not vary sufficiently, at least within the range of leucocyte concentrations which we have studied, to lead to serious error in the use of the absolute variations for comparing the errors of routine analysis, a fact of importance which we shall refer to later in connection with the evaluation of results. In column 4 we have given the probability (P), based on the Chi Square Test of Pearson (1930) (c.f. also, standard works on statistics), of a worse fit to the *normal curve* occuring by chance than that found, assuming the distribution of cells on the unit areas (1 sq. mm.) to be *normal.* "If the magnitude of P is below .05 the test demonstrates that the chances of agreement of the observed results with theory are but 1 in 20, or less, and therefore the validity of the hypothesis may justly be called into question" (Treloar, 1933, p. 32). In only one case (exp. 9) are the odds excessive against a worse fit occurring by chance than that found; but the occurrence of this one case, in which the chances are 1 in 70, is not altogether unlikely in a total of 25 experiments of this type drawn from a normal population. We may therefore conclude that under the above conditions the *normal curve* gives such a reasonably good fit that it may be employed as a means of describing the distribution of leucocytes on unit areas of one square millimeter on the hemacytometer and consequently we are privileged to use in our analysis all accumulated information dealing with distributions which follow the normal law of error.

Errors of the total leucocyte count due to subsampling. In the present analysis of errors each total leucocyte count has been based on the mean of the nine squares on one side of a counting-chamber. Now in a *normal* system the standard deviation of the means (i.e., σ_M) of samples of 9 individuals is given by the formula, $\sigma_M = \frac{\sigma}{\sqrt{9}}$ where σ is the standard deviation of the individuals about the mean of the population (c.f. Mills, 1933). If the *normal law* is the real law describing the distribution of cells on the unit areas and if no further error is introduced by unevenness of mixture of cells and diluting fluid or by filling the chamber by capillarity, it follows that the standard deviations actually observed for the distribution of means of groups of 9 unit areas will correspond, within limits of error of random sampling, to those calculated on the basis of the

TABLE 1

EXPERIMENT NUMBER	A (1) Number of sq. mm. areas counted	A (2) Mean number of cells per sq. mm. (m)	A (3) Standard deviation (σ) (cells per unit area of 1 sq. mm.)	A (4) Probability (P) demonstrated by Chi Square Test—showing fit to *normal curve*	B (5) Number of samples of 9 unit areas each	B (6) Standard deviation of *means* of samples of 9 unit areas, (σ_M). Observed	B (7) Calculated: Gaussian law $\sigma_M = \sigma/\sqrt{9}$	B (8) Calculated: Poisson law $\sigma_M = \sqrt{\frac{m}{9}}$	C (9) Mean total leucocyte count (cells per cu. mm. of blood)	C (10) Mean deviation of total counts (cells per cu. mm. of blood)	C (11) Standard deviation of total counts (cells per cu. mm. of blood)	C (12) Coefficient of variation of total counts (per cent)
1	144	25.39 ±0.36	±4.30 ±0.25	0.300	16	±1.04 ±0.12	±1.43	±1.67	5078	±160	±208	±4.09
2	108	26.16 ±0.51	±5.35 ±0.36	0.862	12	±1.77 ±0.24	±1.78	±1.70	5232	±283	±354	±6.76
3	144	26.93 ±0.37	±4.50 ±0.26	0.736	16	±1.00 ±0.12	±1.50	±1.73	5386	±159	±200	±3.71
4	90	29.22 ±0.59	±5.60 ±0.41	0.351	10	±1.38 ±0.20	±1.86	±1.80	5844	±245	±276	±4.72
5	135	30.16 ±0.39	±4.51 ±0.27	0.634	15	±2.10 ±0.26	±1.50	±1.83	6032	±233	±420	±6.96
6	126	30.93 ±0.50	±5.62 ±0.35	0.528	14	±2.06 ±0.26	±1.87	±1.85	6186	±353	±412	±6.66
7	216	36.28 ±0.41	±6.11 ±0.29	0.902	24	±1.50 ±0.14	±2.03	±2.00	7256	±224	±300	±4.13
8	144	36.24 ±0.46	±5.57 ±0.33	0.760	16	±1.11 ±0.13	±1.85	±2.00	7248	±177	±222	±3.06
9	108	36.33 ±0.57	±5.98 ±0.40	0.013	12	±2.54 ±0.35	±1.99	±2.01	7266	±437	±508	±6.99
10	117	36.45 ±0.47	±5.15 ±0.33	0.852	13	±0.97 ±0.13	±1.71	±2.01	7290	±159	±194	±2.66
11	144	38.05 ±0.45	±5.48 ±0.32	0.683	16	±1.33 ±0.16	±1.82	±2.05	7610	±225	±266	±3.49
12	180	38.13 ±0.38	±5.12 ±0.27	0.291	20	±1.17 ±0.12	±1.70	±2.06	7626	±189	±234	±3.07
13	180	38.18 ±0.37	±5.06 ±0.26	0.672	20	±0.96 ±0.10	±1.68	±2.06	7636	±149	±192	±2.51
14	126	39.40 ±0.42	±4.79 ±0.30	0.564	14	±1.05 ±0.13	±1.59	±2.09	7880	±159	±210	±2.66
15	117	41.16 ±0.61	±6.62 ±0.43	0.111	13	±2.63 ±0.34	±2.20	±2.13	8232	±348	±526	±6.39
16	171	41.47 ±0.36	±4.76 ±0.25	0.481	19	±1.40 ±0.22	±1.58	±2.14	8294	±212	±280	±3.37
17	144	42.88 ±0.42	±5.02 ±0.29	0.555	16	±0.89 ±0.10	±1.67	±2.18	8576	±145	±178	±2.07
18	144	44.14 ±0.47	±5.69 ±0.33	0.485	16	±1.31 ±0.15	±1.89	±2.21	8828	±201	±262	±2.96
19	117	44.97 ±0.54	±5.83 ±0.38	0.434	13	±1.61 ±0.21	±1.94	±2.23	8994	±270	±322	±3.58
20	99	47.30 ±0.44	±4.38 ±0.31	0.730	11	±2.46 ±0.35	±1.46	±2.29	9460	±380	±492	±5.20
21	135	50.80 ±0.58	±6.75 ±0.41	0.267	15	±2.60 ±0.32	±2.25	±2.38	10160	±384	±520	±5.12
22	117	52.38 ±0.51	±5.56 ±0.36	0.166	13	±1.60 ±0.21	±1.85	±2.41	10476	±271	±320	±3.05
23	135	53.38 ±0.46	±5.35 ±0.32	0.607	15	±1.08 ±0.13	±1.78	±2.43	10676	±174	±216	±2.02
24	108	56.62 ±0.65	±6.76 ±0.46	0.780	12	±1.27 ±0.17	±2.25	±2.51	11324	±211	±254	±2.24
25	144	58.56 ±0.52	±6.28 ±0.37	0.770	16	±1.48 ±0.17	±2.09	±2.55	11712	±245	±296	±2.52
Average	135		±5.44 ±0.47	0.541	15	±1.53 ±0.40	±1.81	±2.09		±239 ±55	±306 ±80	
Total	3393				377							

above formula. Thus in experiment 1 of table 1 the mean of 144 unit areas is 25.39 $\pm$0.36 and the standard deviation (σ) is $\pm$4.30 $\pm$0.25; the standard deviation of the means, of the 16 samples of 9 square millimeters each, would be expected from the above formula to be $\sigma_M = \frac{4.30}{\sqrt{9}} = \pm 1.43$ (c.f. Treloar, 1933, p. 56). Actually the value found was $\pm$1.04 $\pm$0.12. Similarly, table 1 (B) gives, for the entire group of experiments, the observed values for the standard deviations of the means (column 6) and those calculated from the normal law of error, i.e. $\sigma_M = \frac{\sigma}{\sqrt{9}}$, (column 7) and from the Poisson law, i.e. $\sigma_M = \sqrt{\frac{m}{9}}$ (column 8). In view of such concordance of the observed and calculated errors as shown in this table we may conclude that, with the proper precautions already outlined, which secure adequate and uniform mixing of cells with the diluting fluid, thus controlling a possible serious source of error, this step as well as that of filling the counting-chamber by capillarity does not contribute greatly to the *variations* of subsampling and that the settling of cells by chance on the ruled field of the hemacytometer is responsible for practically all of the major variations observed in our study. The means and the standard deviations in terms of total numbers of cells per cubic millimeter of blood, obtained by multiplying the observations of the unit areas by 200, are given in table 1 (C) (compare with table 2, discussed below).

Ponder, Saslow and Schweizer (1931) likewise reached the conclusion that most of the variation in leucocyte counts is due to the chance distribution of cells on the counting-chamber, however, the variations which they found for counts based on "one side of a counting-chamber" are on the whole much greater than those which we have found (table 1, column 11). Their statement that "fewer than 600 to 800 cells are not ideally distributed, and the distribution, therefore, cannot be properly represented by a Poisson's series" leads us to believe that some factor, other than the chance distribution of cells on the hemacytometer was prominent in their experiments as a source of variation.

Expression of errors. It is not uncommon to find variabilities expressed in terms of per cent of the mean leucocyte count, i.e., as the coefficient of variation, as has been done in table 1 (column 12) but "a word of warning may not be out of place in reference to the use of this measure. In its interpretation caution must always be exercised not to neglect the fact that its magnitude is partly a function of $\bar{x}$—(i.e., of the mean). Variabilities measured on the same absolute scale should be compared directly as far as possible" (Treloar, 1933, p. 17). It will be observed from table 1 that the variabilities measured on the same absolute scale do not differ appreciably within the range of the total leucocyte counts included

in this study, whereas the coefficients of variation differ significantly with different magnitudes of the total leucocyte count (compare expts. 7 and 25—table 1, C), therefore, the direct comparison of values on the absolute scale is distinctly more accurate in the interpretation of errors in the leucocyte count.

II. THE FINAL ERROR OF ANALYSIS IN THE DETERMINATION OF LEUCOCYTE COUNTS (THE COMPOUND VARIABLE). We have determined the frequency distribution of the *compound* variable when all possible sources of variation were included in the routine procedure.

Physiological factors in the compound variable. For technical reasons we have adopted the deep finger "stab" as a constant technique for obtaining blood samples for our physiological studies and have included in our *final compound error of blood counting* the added factor of sampling from a finger "stab" wound. As pointed out by Shaw (1926), capillary blood may show marked deviations from the systemic (arterial) blood if the capillaries are in a state of constriction, whereas if dilatation be assured by friction or by heat, then the blood obtained from this dilated region gives leucocyte concentrations consistently identical with arterial blood taken from any point in the systemic circulation. Our repetition of Shaw's experiments has confirmed the constancy of blood samples taken under the above conditions of dilatation, hence we have adopted the following routine procedure which accomplishes the purposes of his method: In as much as capillary dilatation, caused either by friction or by heat, yields a copious flow of blood in most cases when the "stab" is made, and since several drops of blood are almost invariably lost before the sample can be taken under these conditions, it may be assumed that the "capillary" blood would all be washed out with the first few drops that flow and that the later drops, which are in reality obtained after dilatation due to puncture has set in, represent practically pure arterial or systemic blood (c.f. Krogh, 1929) hence, we have compared the samples obtained from a "stab" wound without heat or friction, but from which the first five drops of blood have been wiped away, with similar samples taken after vasodilatation produced either by heat or friction. All experiments of this type have yielded the same uniformity of results under the three different methods of obtaining systemic blood. In wiping off the first drops a film of blood may be left on the finger, this quickly dries and hastens clotting in the succeeding drops, a difficulty which was easily overcome by wiping each drop of blood with a gauze sponge moistened (not wet) with 5 per cent oxalic acid, followed immediately with a dry sponge before the drop to be sampled was allowed to form. This method has a very obvious advantage particularly noted in subjects who are having blood taken for the first time, and in whom vasoconstriction may occur as a reflex or emotional response to the "stab" even after the capil-

laries have been previously dilated by friction or by heat. The emotional response of some individuals is very striking and indeed we have observed four subjects who fainted immediately after the "stab" was made. Figure 1 (A) shows a plethysmogram taken from the arm on the opposite side of the body from which a "stab" was feigned in a subject who had previously fainted when a "stab" was actually made; his peripheral vasoconstriction was evidently maximal when a "stab" was faked with the blunt end of a cataract knife at 4, since the actual "stab" at 5 produced no additional vasomotor effect and the wound did not begin to bleed until almost a minute later. Figure 1 (B) shows a similar experiment on a subject who characteristically failed to bleed immediately after a "stab"

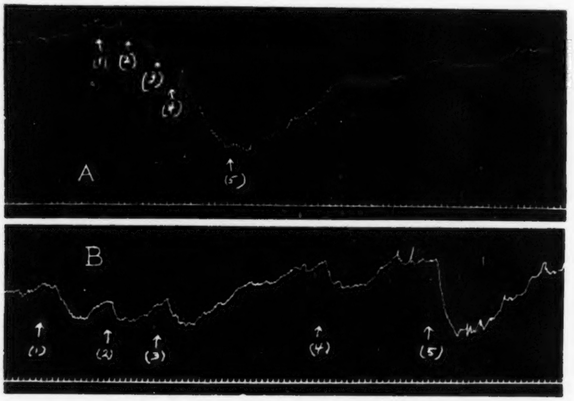

Fig. 1. (A. and B.) Plethysmograms from two subjects record volume change of arm on side of body opposite that on which finger "stab" was made. Downward movement represents a decrease in arm volume (vasoconstriction). *1.* Knife (cataract) laid on table before subject. *2.* Finger prepared for "stab." *3.* Feigned "stab"; finger not touched with knife. *4.* Quick jab with blunt end of knife. *5.* "Stab" actually made. Time in six second intervals.

was made but who usually began to bleed quite freely several seconds (20–40) later. Such reactions as these have been quite common among our subjects, especially those who were participating for the first time. They may become less marked, however, after repeated participation in the experiments, a fact which emphasizes the psychic element in the reaction. That the more vigorous reactions, e.g., fainting, may be associated with definite alterations in the leucocyte content of the systemic blood is suggested by a few of our experiments but the milder peripheral vasoconstriction probably affects the count only locally, as was demonstrated by Shaw (1926) in a study of closely related reactions. The temporary peripheral vascular reaction usually lasts only 20 to 40 seconds

after which an initial vasoconstriction is followed by active vasodilatation. During the latter phase the blood flows freely if an adequate "stab" has been made and it is during this phase that the sample should be taken. The wound may bleed slowly or may not bleed at all during the first 20 to 40 seconds due to vasoconstriction; it is a natural inclination during this phase to squeeze the finger, a procedure which should not be resorted to since it expresses tissue juices thus hastening clotting of the blood and preventing free flow later. In so far as we have been able to determine, slight pressure, sufficient to open the wound and to impede venous return in the finger thereby increasing the flow of blood from the wound, does not alter the leucocyte content of the blood if it flows freely under this condition and if the pressure be applied at some distance from the wound.

By considering the above factors, we have adopted the following desirable procedure which has been followed in obtaining the samples for the study of the *compound error* previously referred to. First a clean "stab" has been made with a sharp cataract knife following the suggestion of Garrey and Butler; second, at least five large drops of blood were wiped off with sponge moistened with oxalic acid as described above before taking the sample which has not been taken sooner than 20 seconds after the "stab" was made; occasionally one meets subjects with cold pale fingers who may not bleed freely with this technique in which cases we have warmed the finger tip with water at 39° to 40° for at least 1 minute before sampling in the usual way; third, a check sample has been taken in a second pipette from the same wound but from a fresh drop of blood. It is not very likely that an appreciable alteration in the systemic blood will take place between the filling of the two pipettes so that they may be considered as having been filled with identical samples of blood.

Technical factors in the compound variable. In order that the technical factors previously considered might be adequately represented in this analysis of the *compound variable*, each of the pipettes was filled by a different observer and one total count made from the contents of each pipette by each of two observers. In this way four independent counts were made for each determination of the leucocyte concentration. In view of the markings of the hemacytometer (Neubauer ruling) it is more desirable for routine purposes to count the cells on the four corner and middle squares (1 sq. mm. each) of the counting field on *both* sides of the counting-chamber, that is, 10 square millimeters in all for each leucocyte count. This number of unit areas not only simplifies calculation of the total leucocyte count but slightly increases the accuracy of the final result by increasing slightly the accuracy of the mean (number of cells per square millimeter) over that obtained with the 9 square millimeter area on one side of the chamber. The fact that one unit determination is made up of two subsamples from the same pipette should not alter the results since

we have seen that most of the error of subsampling can be accounted for by the chance distribution of cells on the hemacytometer when the precautions noted under that heading are taken. As additional evidence of this fact, Purdy (1925), in his excellent study of the error of counting leucocytes with apparatus of special design, has shown that equally good results are to be expected by counting any given number of subdivisions of the counting field, whether the counts be made on a single preparation or on several preparations, from the same bulk dilution of blood.

Experimental. Each group of four counts for each leucocyte determination performed according to the above technique, has embodied the following combination of the elementary variables: 1, two samplings, including physiological factors if present; 2, two dilutions; 3, eight "subsamplings," 4, the personal variations of two individuals; and 5, two pippettes and two counting-chambers. Each experiment involves the determination of the deviations of each of these counts from the mean of the group, i.e., the errors observed when four counts are made on the same sample of blood.

Results and analysis. For the sake of analysing the compound error of blood counting it would have been more desirable to base each experiment on 10 or more blood counts, but since we have sampled from a flowing stream of systemic blood, and, considering the possibility that the leucocyte content of the blood may change significantly as a result of physiological factors, within the time necessary to acquire so many samples, we have filled only two pipettes in rapid succession with the assumption that the leucocyte content of the systemic blood will not vary appreciably during this short time. Obviously physiological variations, if interjected, would obscure the true *errors of analysis.* In order to compensate for the paucity of counts in each individual experiment, we have considered a large number of experiments, i.e., 627,[2] involving a total of 2508 counts (each based on the counting of 10 unit hemacytometer areas of one square millimeter). The errors in the total counts have been assembled into groups, being classified according to the magnitude of the mean of the four leucocyte counts for each sample of blood; the errors represent *deviations* of each of the four counts about this mean. Since we have found no correlation between the magnitude of the errors and the magnitude of mean leucocyte counts included within limits of a given class interval, we have treated the errors falling within each class interval as though they had been obtained on a single sample of blood with a leucocyte concentration equal to the mean of the class interval group.

[2] The 627 blood samples have been obtained from approximately 200 different subjects and have been taken at various times of the day under various normal physiological conditions. We have not attempted to control these conditions since we have been interested only in observing the errors obtained in many different samples of blood regardless of the individual or his physiological state.

The statistics describing the distribution of these deviations (i.e., errors) for each class interval group have been given in table 2. From this table it may be seen that the errors do not differ significantly from the errors due to subsampling alone which have been similarly expressed in table 1 (C). This finding, considering the fact that two pipettes filled from different drops of blood were employed for each blood analysis, eliminates from our consideration the probability of disturbing factors being introduced in the process of sampling from a finger stab.

It may be confidently stated, therefore, that with the technique which we have employed in this work all other sources of variation in the routine

TABLE 2

Errors of routine analysis observed at different levels of the total leucocyte count within physiological limits

Each count has been based on the counting of 10 unit hemacytometer areas of one square millimeter: Four counts have been made for each sample of blood.

LEUCOCYTE CONTENT OF BLOOD SAMPLES (CELLS PER CU. MM. OF BLOOD)	NUMBER OF BLOOD SAMPLES	NUMBER OF COUNTS	MEAN DEVIATION OF ERRORS (CELLS PER CU. MM. OF BLOOD)	STANDARD DEVIATION OF ERRORS (CELLS PER CU. MM. OF BLOOD)	COEFFICIENT OF VARIATION (PER CENT)
Between 3000 and 4000	8	32	±219	±269	±7.68
Between 4000 and 5000	103	412	±165	±210	±4.66
Between 5000 and 6000	196	784	±177	±234	±4.25
Between 6000 and 7000	158	632	±195	±259	±3.98
Between 7000 and 8000	72	288	±235	±304	±4.05
Between 8000 and 9000	49	196	±265	±342	±4.02
Between 9000 and 10000	20	80	±261	±334	±3.51
Between 10000 and 11000	8	32	±284	±368	±3.50
Between 11000 and 12000	3	12	±362	±473	±4.11
Between 12000 and 13000	3	12	±200	±231	±1.85
Between 13000 and 14000	2	8	±263	±294	±2.18
Between 14000 and 15000	5	20	±268	±320	±2.20
Total....................	627	2508			
Average................			±241 ±35	±303 ±46	

procedure of leucocyte counting can be and have been adequately controlled so that the variations due to them are reduced to a position of minor and negligible importance and that the observed variation in our total leucocyte counts even when sampling from a finger stab wound are to be accounted for by the chance distribution of the cells on the ruled field of the counting-chambers. It does not necessarily follow, however, that the factor of chance distribution of cells on the counting-chamber is always the only source of significant error, for in ordinary routine analysis it is especially easy for other factors, i.e., uneven mixture of cells and diluent,

filling the counting-chamber, clumping of cells due to partial clotting of blood, local physiological factors in sampling from "stab" wound etc., to creep in and to interfere greatly with the accuracy of the results. If appreciable errors enter in occasionally due to any of these causes, the curve of errors instead of being *normal* will be leptokurtic, i.e., a higher per cent of the errors will fall toward the tails of the curve and may even extend the tails, or the range of the errors, significantly. Student (1927) has emphasized the fact that we are all fallible and prone to blunders, hence, "many if not most routine analyses have a leptokurtic error system," thus it is only natural to repeat and discard results which are not concordant, i.e., vary over a wide range. He has therefore developed a method for increasing the accuracy of a mean in a leptokurtic error system, by a systematic procedure of repetition and rejection of outlying results. If in the counting of leucocytes greater errors are met with than would be expected from the chance distribution of cells on the hemacytometer we see no reason why repetition and rejection as described by Student cannot be used as a means of increasing the accuracy of the results; however, the "blunder" should be proven to be purely technical by repetition of the count from the same bulk of diluted blood from which the "blunder" was made, or, the entire pipette may be discarded because of clumps, etc., but care should be taken not to discard results which might have been altered by causes other than faulty technical procedures.

SUMMARY AND CONCLUSIONS

1. The error of total leucocyte counting is a resultant of several elementary variables. The major source of variation has been shown to be inherent in the technical procedure of "subsampling," i.e., the combined factors of mixing the cells and diluting fluid, filling the counting-chamber and settling of cells by chance on the ruled field of the counting-chamber. Methods have been described by which these may be controlled to such an extent that only one of them, namely, the chance distribution of cells on the ruled field of the counting-chamber accounts for most of the variation observed. Under the conditions described the distribution of leucocytes on unit areas of one square millimeter is sufficiently close to the *normal probability curve* that information relating to the normal law of error is available in the study of the distribution on these unit areas and consequently in the evaluation of total leucocyte counts calculated therefrom.

2. Where the method has been uniform, variabilities (i.e., errors) of leucocyte counts (within the limits of normal physiological variation) are more accurately compared directly on an absolute scale than in terms of per cent since the latter is partly a function of the mean leucocyte count, which in itself may vary more than 100 per cent in different indi-

viduals or even in the same individual under different physiological conditions.

3. When proper precautions are taken sampling from a finger stab wound does not introduce additional errors and the final error of total leucocyte counting (i.e., the *compound variable*) does not differ essentially from the error due to "sub-sampling" alone.

The average error of routine analysis when total leucocyte counts are based on 10 unit hemacytometer areas of one square millimeter each and when the dilution of the blood is 1 to 20 has been found, empirically, on analysis of 2508 counts, to be $\pm 241 \pm 35$ cells per cubic millimeter of blood. The magnitude of this error does not differ appreciably at the different levels of the leucocyte count included in this study as is shown by the low value of its probable error. The standard error of analysis with this method has been found to be $\pm 303 \pm 46$ and the probable error $\pm 204 \pm 31$ cells per cubic millimeter of blood.

4. Certain steps in the technical procedure, which can be controlled, may occasionally introduce significant errors into the results, therefore, it is desirable to perform analyses in such a way as to permit an accurate check on all sources of variation and to determine the error and probable significance of individual analyses rather than to assume a blanket limit of error for the method, an assumption which may give a false sense of accuracy in some cases while obscuring greater accuracy in others.

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THE LEUCOCYTE COUNT OF YOUNG MALE ADULTS OBSERVED AFTER A PERIOD OF REST AND DURING MILD ACTIVITY IN THE EARLY MORNING[1]

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Leucocyte counts from different individuals taken at various times of the day and under various normal physiological conditions have been reported to cover a very wide range. It is recognized that these variations are to be accounted for at least in part by variations in physiological state of the individual and Naegeli (1931) therefore emphasizes the precaution that counts should be made in the early morning under conditions of physiological rest. Garrey and Butler (1929) found that when their subjects were examined in the basal state[2] the counts fell within the narrow "band" of from 5000 to 7000 cells per cubic millimeter of blood. Arneth (1920) likewise reports that the resting morning count lies between 5000 and 6000. More recently, however, Schweizer (1933) has reported leucocyte counts which ranged from 4700 to 11,500 when taken in bed in the early morning after a full night's rest. She also contradicts the previous conclusion of Garrey and Butler that there is a definite and significant difference between the "basal level" and the "activity level" of the leucocyte count.

The concept that there is a sharp and distinct difference between the leucocyte count taken at rest and during random activity has been the source of considerable controversy. It is for this reason that we have undertaken a reinvestigation and extension of the previous studies of Garrey and Butler.

Technical. The technical procedures used in this investigation were precisely the same as those previously described by Bryan, Chastain and Garrey (1935) (1934). Each leucocyte count herewith presented repre-

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[2] The state which they considered as basal in their experiments was the same that is used clinically in the determination of basal metabolism. However, they observed only a slightly higher leucocyte count taken after one hour of rest in the afternoon and therefore applied the term "basal count" to samples of blood taken after one hour of rest at any time of the day. In this report we shall use the term basal only in the strictest sense as it is applied clinically.

sents the average of four simultaneous leucocyte determinations, each based upon the counting of 10 unit areas of one square millimeter each, or a total of 40 unit areas counted for each sample of blood.

Leucocyte counts taken during rest in the early morning after fasting over night (basal state) as observed on different days in a given individual. Stetson (1927) has shown that appreciable variations in the total white blood cell count occur from day to day even when the subjects are observed under conditions of minimal metabolism. "The variations were often

TABLE 1

The average leucocyte count and the range of variation of the leucocyte count observed on different days in eighteen young male adults during rest in the early morning, after fasting over night

SUBJECT	NUMBER OF OBSERVATIONS	MEAN LEUCOCYTE COUNT	MINIMAL COUNT	MAXIMAL COUNT	DIFFERENCE BETWEEN MAX. AND MIN. COUNTS
1	6	6219	4960	7435	2475
2	5	5651	5020	7010	1990
3	5	5737	5210	6770	1560
4	4	4965	4230	5765	1535
5	5	6963	6390	7375	985
6	8	5594	5025	6390	1365
7	7	7217	6375	8510	2135
8	6	7538	7050	8005	955
9	6	5113	4735	5605	870
10	5	5852	5227	6905	1678
11	6	4164	3820	4540	720
12	15	5320	4445	6095	1650
13	13	5501	4740	6250	1510
14	19	4268	3195	6155	2960
15	11	6223	5105	8000	2895
16	4	3907	3405	4400	995
17	4	4564	3905	5460	1555
18	4	6345	5810	6849	1039
	Total 133	Mean 5618	Minimum 3195	Maximum 8510	Mean diff. 1604

greater than 1000 and even as great as 2400 cells per cubic millimeter." The extreme variation which he found for any one person during the entire period of observation (several months) was 4125 while the least maximal variation was 1475. He further states that "the degree of these fluctuations in the number of white blood cells under conditions of minimal metabolism is not as great as has been observed in relatively inactive persons throughout the day."

We have studied the leucocyte count on four or more occasions in 18

different young healthy male adults during rest in the early morning (7:30 to 9:30 a.m.) after fasting over night. The results of our observations are presented in table 1 which shows the average, the minimal and the maximal leucocyte counts and the difference between the highest and lowest count observed for each subject. The greatest maximal variation observed for any one subject of this group is 2960 cells, while the average maximal variation for the entire group is 1604 cells. These results correspond very closely to those reported by Stetson (1927); thus we may conclude with the latter, that the leucocyte count even under conditions of minimal metabolism may show significant variations from day to day in the case of a given individual. The extreme limits of variation observed for all basal experiments on all subjects of this group are: highest count 8510, lowest count 3195, while the average for the entire group is 5618.

TABLE 2

Cumulative distribution of 116 leucocyte counts obtained on 75 young male adults during rest in the early morning

Classified according to the number of cells per cubic millimeter of blood (Cumulated upward)

MAGNITUDE OF LEUCOCYTE COUNT (NUMBER OF CELLS PER CU. MM. BLOOD)	NUMBER OF COUNTS	PER CENT
Less than 3000	1	0.86
Less than 4000	10	8.62
Less than 5000	39	33.62
Less than 6000	75	64.65
Less than 7000	103	88.79
Less than 8000	113	97.41
Less than 9000	116	100.00

Leucocyte counts taken during rest in the early morning after fasting over night (basal state) as observed in 75 different male subjects. Having found that significant variations may occur in the case of a single individual on different occasions, we made repeated observations on each subject—with a total for all subjects of more than 300 leucocyte counts (each an average of four simultaneous analyses) taken under basal conditions—but in our statistical analysis, which follows, we have included any one subject not more than once in a given class interval group; that is to say, if all of the repeated experiments gave counts which fell within limits of the same class interval (e.g., 5000 to 6000) the subject has been represented only once in the frequency distribution (i.e., fig. 1 and table 2), whereas if the counts fell in more than one of the class interval groups he has been represented more than once in the frequency distribution. Such a frequency distribution is more representative of the magnitudes of the leuco-

cyte counts in the population of which our experiments constitute a sample than would be a distribution of single observations on each subject, in view of significant individual variations from day to day, or than would be a distribution of the averages of several observations on each subject, which procedure obviously might give an erroneous idea of the range of the individual counts. The procedure which we have used is equivalent to studying a larger group of subjects, for if a given subject shows counts of different magnitudes on different occasions he may rightly be considered as a different individual on each occasion. Our observations have been repeated more often in some subjects than in others, especially in the case of those on whom we have found our extreme or limiting values of the leucocyte count under basal conditions. The results obtained in these repetitions give us confidence that the limiting values which we have found are the actual counts for the individual under the conditions employed and are not accidental or due to "blunders" in technique, etc., and furthermore that these limits are not too narrow for the group of individuals which we have studied. Since any given individual has been represented not more than once in a given class interval group and each subject has an equal chance of falling in any given class group, the relative influence of each subject on each class interval group, and therefore on the distribution of magnitudes as a whole, has remained the same. It has been impossible to obtain exactly the same number of observations on each subject and had we included all observations on all subjects in our frequency distribution, obviously, humps would occur in the curve at class intervals having the most repetitions especially at the tails of the curve since more repetitions have been made in these groups; however, the limits would not be changed.

In recent publications on the leucocyte count emphasis has been placed on the range of variation rather than on the average leucocyte count which was formerly taken as the basis of comparison. However, neither of these measures alone is sufficient since the average gives no idea of the limits of variation while the range gives entirely too much weight to extreme values. The standard deviation gives a measure of dispersion, or scatter, but does not show the type of frequency distribution with which we are dealing. In addition to the statistic measures, we have presented our results in the form of a simple frequency distribution in order that the type of frequency distribution with which we are dealing and the relative frequency of leucocyte counts at different magnitudes may be more readily seen (vid. fig. 1, curve *A*; and table 2). In figure 1, curve A, we have shown the curve of frequency of 116 leucocyte counts (each an average of four simultaneous determinations) obtained on 75 different male subjects between the ages of 18 and 30 years; the counts have been classified as described above. This curve of distribution is

seen to be quite symmetrical and to be an excellent fit to a *normal* curve as was determined by the Chi Square Test of Pearson (1930) (c.f. also standard works on statistics), from which the probability (P) was found to be 0.942. The mean leucocyte count for this entire group of observations is 5610, while the range extends from the lowest count of 2700 to the highest count of 8600; the standard deviation of the counts about the mean is ±1205 and the coefficient of variation (V) is 21.5. The frequency distribution of the 116 leucocyte counts is shown cumulatively in table 2 which gives the portion of the total number of observations below each class interval limit thus offering more readily a basis of comparison of these results with those of other investigators.

Shaw (1927) has published the frequency distribution of total leucocyte counts which he made "between 9:00 a.m. and 10:00 a.m. from 116

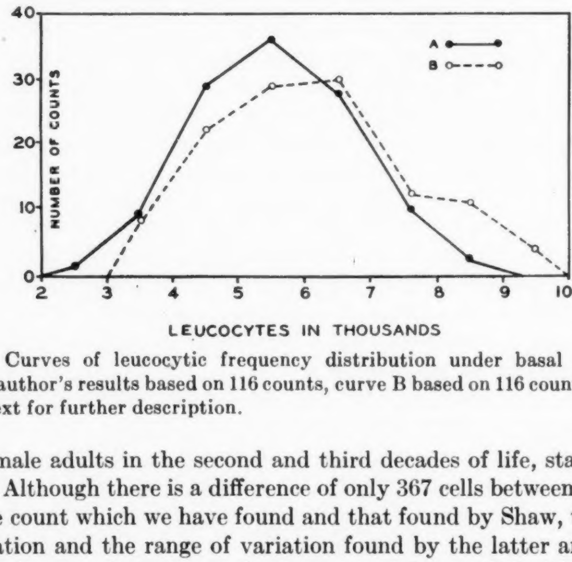

Fig. 1. Curves of leucocytic frequency distribution under basal conditions. Curve A, author's results based on 116 counts, curve B based on 116 counts by Shaw. Consult text for further description.

healthy male adults in the second and third decades of life, starved over night." Although there is a difference of only 367 cells between the mean leucocyte count which we have found and that found by Shaw, the standard deviation and the range of variation found by the latter are slightly greater. A curve drawn from the frequency table published by Shaw is shown in figure 1 (B) in comparison with our own results (curve A). It will be observed that Shaw's distribution is definitely skewed toward the higher values of the leucocyte count, which indicates that the chances of obtaining leucocyte counts of the higher magnitudes are greater in Shaw's experiments due probably to some factor or factors determined by the conditions of observation.

Leucocyte counts taken during mild activity in the early morning, after fasting over night, as compared with leucocyte counts taken after one hour of rest in the same group of subjects. It is a well established fact that vigorous

muscular exercise increases the leucocyte count of the circulating blood (Martin, 1932; Garrey and Butler, 1929; Zirm and Bauermeister, 1933; Ponder, Saslow and Schweizer, 1931) and that the number of cells is greater the more severe the exercise (Raisky, 1932; Martin, 1932). On the other hand it has been frequently denied that muscular activity of a *mild* degree ("random activity") increases the number of leucocytes in the circulating blood (Shaw, 1927; Schweizer, 1933; Ponder, Saslow and Schweizer, 1931). Jones, Stephens, Todd and Lawrence (1933) studied the leucocyte count in six subjects who "were allowed to be up and around the laboratory with mild exercise for a short period," and, since "the curves of four of these subjects differed in no essential way from those of the nine subjects who were kept recumbent" they conclude that their results "tend to indicate that short periods of activity in normal subjects may not be associated with any corresponding changes in the total number of white blood cells. Whether the marked variations which did occur in two of our subjects when mild activity was allowed were due to the mild infections—(which it is stated that they both had)—or to the exercise cannot be stated." It is of interest to note that the mean leucocyte count of their four "normal" subjects during activity is slightly higher than the mean of their nine recumbent subjects, i.e., 6364 as compared with 5332, a difference of 1032 cells. As these authors have pointed out, this difference is not significant statistically in comparison with the probable errors of their means; however, this number of observations is too small to permit general statistical conclusions relating even to the population from which their experiments were drawn.

It is perfectly obvious that the actual amount of exercise involved in "random activity," "mild activity," "ordinary daily routine," etc. is a relative matter and that individuals pursuing different types of activity may yield quite different results. In order that the activity in different individuals which we have studied might be at least grossly comparable, we have established the following conditions for our observations: Since all of our subjects have lived within a few blocks of the campus, their leisurely walk to our laboratory in the early morning, after fasting over night, has been considered as "mild activity." Blood samples were obtained immediately upon their arrival at the laboratory, this being labeled as the "activity count" and again after they had rested in bed for one hour, the latter being labeled as the "rest count." The results which we have obtained in 100 experiments of this type on 50 male adults are presented in figure 2 in which the rest and activity counts have been grouped in separate frequency distributions and plotted accordingly. The curve for the "rest counts" (A) though slightly skewed, is seen to be a reasonably good fit to a *normal* curve, the chances being about 50/50 that a worse fit might be obtained due to random sampling ($P = 0.547$); this curve

does not differ significantly from that shown in figure 1 (A) for counts taken under similar conditions. On the other hand, the curve of the "activity counts" (B) is quite markedly skewed and tails out toward the higher values of the leucocyte count; the frequencies of the higher counts are accordingly much greater than was found for the rest counts and much greater than could occur in a population normally distributed as a result of random sampling alone. It thus becomes evident that some factor or factors associated with random activity of the type which we have studied, in some instances, in some subjects at least, causes an increase in the total leucocyte count. Although these results do not confirm the existence of definite and distinct zones of variation ("bands") for the conditions of rest and activity, it is unquestionable that the range of variation is greater and that the upper limit of variation is higher during

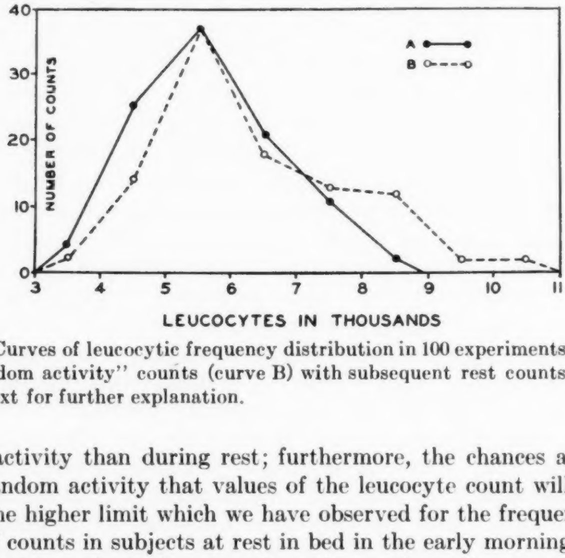

Fig. 2. Curves of leucocytic frequency distribution in 100 experiments comparing mild "random activity" counts (curve B) with subsequent rest counts (curve A). Consult text for further explanation.

random activity than during rest; furthermore, the chances are greater during random activity that values of the leucocyte count will be found toward the higher limit which we have observed for the frequency distribution of counts in subjects at rest in bed in the early morning.

Clearly this analysis does not show the results to be expected in any single subject, i.e., the chances that the "rest count" will be lower than the "activity count" or vice versa. This expectancy can be readily seen when the experiments are classified in class interval groups according to the magnitude of the "activity count" as we have done in table 3 which shows for each class interval group the mean leucocyte count obtained during activity and during rest, the mean difference between these counts, the standard error of the mean differences, and the probability that the mean rest count would be lower than the mean activity count on repetition of these observations in the same population of subjects. (The proba-

bilities have been determined by the use of tables of the probability integral for the *normal* curve (c.f. Pearson, 1930) where the number of experiments is greater than 10—c.f. Student, 1908—and by the use of the special tables of Student (1908) where the number of experiments in the class interval group is less than 10.) Although the data given in table 3, when considered as a whole indicate that the difference between the mean activity count and the mean rest count is not statistically significant (the probability that the rest count is actually lower is 0.785), yet when one considers the class interval groups separately, all groups above the group 6000 to 7000 show a definite and significant tendency for the mean leucocyte count to be lower after one hour of rest; the magnitude of this

TABLE 3

Summary of 100 experiments on 50 young male adults in whom the leucocyte count was observed during mild activity in the early morning immediately before a period of rest and again after one hour of rest

The experiments are classified according to the magnitude of the initial activity count. Class interval = 1000. The column mark P represents the probability that the mean rest count is actually lower than the mean activity count (see text).

(1) CLASS INTERVAL (NUMBER OF CELLS PER CU. MM.)	(2) NUMBER OF EXPERIMENTS	(3) MEAN COUNT DURING MILD ACTIVITY	(4) MEAN COUNT AFTER ONE HOUR OF REST	(5) MEAN DIFFERENCE (3-4)	(6) STANDARD ERROR OF DIFFERENCE	(7) P
3000 to 4000	2	3587	3697	+110	±255	0.371
4000 to 5000	14	4650	4663	+13	±172	0.492
5000 to 6000	37	5478	5083	−395	±61	0.820
6000 to 7000	18	6378	5697	−681	±50	0.997
7000 to 8000	13	7436	6433	−1003	±52	0.993
8000 to 9000	12	8298	6971	−1327	±244	0.942
9000 and above	4	9901	7239	−2662	±81	1.000
All experiments......	100	6256	5596	−660	±83	0.785

negative difference and its incidence in individual experiments are greater the higher the initial activity count. When the initial activity count is less than 6000, positive differences are not infrequent and may predominate in the lower class interval groups due to chance; however, the nature of these chance variations cannot be considered with a high degree of probability as characteristic for the group in view of the relatively large standard errors of the differences.

In view of the fact that we have not observed in 100 experiments on 50 subjects, values above 10,500 for the leucocyte count in the early morning during random activity, we have increased the initial count in a few subjects through exercise (standing, running or riding a bicycle). Most of the subjects of this group were selected from those who charac-

teristically have shown resting counts above 6000. Table 4 shows the individual counts and the differences observed in this group. In all cases the leucocyte count after having been increased through exercise dropped back during the hour of rest to within the range which we have observed for resting subjects in the early morning.

Considering the results of the above observations we might explain the previous conclusion of Garrey and Butler (1929), all of whose subjects had activity counts of at least 8000 (and the results obtained by them were to be expected), namely, that the leucocyte count was invariably lower after one hour of rest; and contrariwise for the experiments of Ponder,

TABLE 4

Experiments showing the leucocyte counts observed in 10 different subjects during the early morning (1) during mild activity, (2) after severe exercise (riding a stationary bicycle exp. 1 to 5; standing running exp. 6 to 10), (3) after one hour of rest in bed, (4) the difference between counts of severe exercise and rest, and (5) the difference between counts of mild activity and rest

EXPERIMENT NUMBER	(1) INITIAL COUNT DURING MILD ACTIVITY	(2) COUNT AFTER SEVERE EXERCISE	(3) COUNT AFTER ONE HOUR OF REST IN BED	(4) DIFFERENCE BETWEEN COUNTS OF SEVERE EXERCISE AND REST (2-3)	(5) DIFFERENCE BETWEEN MILD ACTIVITY AND REST COUNTS (1-3)
1	10469	13286	7481	−5805	−2988
2	6482	11565	6026	−5539	−456
3	6458	10070	6127	−3943	−331
4	4750	9805	4412	−5393	−338
5	9138	15044	7800	−7244	−1338
6	6544	9970	5560	−4410	−984
7	8376	13082	7176	−5906	−1200
8	7432	10410	6830	−3580	−602
9	7328	11422	6114	−5308	−1214
10	8772	11240	6714	−4526	−2058
Averages...	7575	11589	6424	−5165	−1151

Saslow and Schweizer (1931), none of whose subjects had activity counts above 6000, our experimental analysis indicates that one would not expect a significant difference between counts taken during activity and during rest. However, our findings do not explain the results of Schweizer (1933) who found "basal" leucocyte counts ranging all the way from 4700 to 11,500 with an average of 7502 cells per cubic millimeter of blood, and activity counts on the same group of subjects ranging from 3200 to 11,500 with an average of 7356. She concluded that the "counts taken during activity were not uniformly higher than those during complete rest, and just as frequently both counts were substantially the same, or the count during activity was lower than that during rest." It is of interest to note

that 26 per cent of the "basal" leucocyte counts obtained by Schweizer are above the highest value which we have found for male subjects, i.e., 8600, and that 16 per cent are above the highest counts obtained by Shaw (1927) on 116 healthy male subjects during the early morning, i.e., 9650. When Schweizer's "basal" counts are grouped into a frequency distribution similar to those above it is seen to be much more skewed and to tail out more markedly toward the higher values than the distributions found either by Shaw or by us even during activity; furthermore the curve shows a double peak the first and larger of which drops off in the class interval group 8000 to 9000 (our highest group) while the second and smaller peak is maximal at the class interval group 9000 to 10,000. A similar double peaked curve is seen in the distribution of her counts observed during activity.

Although we cannot explain the difference in the results which we have obtained and those found by Schweizer, we may point out some differences in the conditions of observation as well as some probabilities which might account for this difference: 1. Our observations, as well as those of Shaw (1927) have been made on healthy male adults in the second and third decades of life, while those of Schweizer have been made on both male and female subjects. It is generally stated that there is no significant difference in the leucocyte counts of male and female subjects; however, we have observed at least one female subject who is healthy in every way, except possibly for a slight obesity, but who characteristically has a leucocyte count, under basal conditions, 1500 to 2000 cells above the upper limit which we have found for healthy male subjects. The remainder of the female subjects whom we have observed (i.e., 75 counts on 25 different subjects) show leucocyte counts which fall within the limits observed for male subjects. 2. The number of subjects whom we have studied may be too few to give accurate conclusions of the limits as they relate to the population as a whole. 3. The population of which our observations constitute a sample may for some unknown reason differ fundamentally from the population studied by Schweizer. In consideration of this possibility it is well to state that most all of our subjects have been medical students who were familiar with the problem and with the technical procedures involved, a point in favor of greater emotional stability of our group of subjects during the experiment. 4. Certain uncontrolled technical factors or physiological factors met with in the process of sampling, such as the emotional response of unaccustomed subjects to the finger stab (Bryan, Chastain and Garrey, 1935) may have accounted for the greater proportion of high counts obtained by her.

SUMMARY AND CONCLUSIONS

1. We have confirmed the conclusion of Stetson (1927) that the leucocyte count, even under conditions of minimal metabolism, may show

significant variations from day to day in the case of a given individual. The greatest maximal variation which we have observed in any one subject among 18 young male adults is 2960 cells, while the average maximal variation is 1604.

2. The limits of variation which we have found for the leucocyte count taken during rest in the early morning, after fasting over night, in more than 300 experiments on 75 different young male adults are: lowest count 2700, highest count 8600. The mean count for this group of subjects is 5610. The frequency distribution of the counts under this condition was found to be an excellent fit to the *normal* curve. This indicates that the conditions of observation are quite comparable in all of the 75 different subjects and that the results are free from extraneous influences, whether technical or physiological, which would tend to increase the range of variation and cause the positive skewing such as has been observed during mild activity and which is characteristic of the data reported in some studies on the leucocyte count under resting conditions in the early morning.

3. The existence of definite and distinct "bands" of variation for leucocyte counts taken during rest and during mild activity has not been confirmed. Nevertheless, the range of variation is on the whole greater during mild activity and there is a definite tendency for the count to be higher in some subjects, especially those who characteristically show the higher counts during rest.

4. We have not obtained counts under conditions of rest in the early morning of the higher magnitudes reported by Schweizer (1933). Some probabilities which might account for this difference in results have been discussed.

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THE INFLUENCE OF SODIUM FLUORIDE UPON THE BASAL METABOLISM OF THE RAT UNDER SEVERAL EXPERIMENTAL CONDITIONS[1]

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Previous studies in this laboratory by Phillips et al. (1934) have shown that fluorine toxicosis influenced tissue respiration, caused pathological changes to occur in the thyroid gland, and increased the fluorine content of the thyroid gland in cattle (Chang, 1934). These observations suggested that the thyroid gland might be involved to a considerable extent in the development of fluorine toxicosis. In an attempt to pursue this suggestion further an experiment was planned to determine the influence of NaF ingestion upon the basal metabolism of the rat under several experimental conditions.

Goldenberg (1927), Gorlitzer (1932), and more recently Raveno (1934) have reported the amelioration of the symptoms of thyrotoxicosis by the oral administration of fluorides or by the use of warm water baths containing HF. This evidence appears to indicate that fluorides can be used therapeutically to control thyrotoxicosis.

On the other hand experience in this laboratory has shown that fluorine ingested in any form results in a series of subtle physiological reactions which are distinctly harmful to the function and health of experimental animals. It has led us to the view that fluorine should be used with extreme caution, if at all. It appears that more experimental work is needed to establish the effect of fluorine on basal metabolism and the conditions under which it can be used.

Experimental. Vigorous and healthy young mature rats were selected for this experiment. They were fed basal ration A previously described by Lamb et al. in 1933. The ration contained yellow corn 55.75 parts, wheat middlings 24 parts, linseed oil meal 12 parts, alfalfa meal 3 parts, tankage 2 parts, steam bone meal 2 parts, limestone 0.5 part, cod liver oil 1.0 part, and iodized salt 0.5 part.

Since much of the information desired depended upon basal metabolism determinations it seemed best to use a limited number of animals but so

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

arrange the allotment and periods of observation in such a manner that weekly data were obtained. Four lots containing 3 and 4 animals each were fed the basal ration A with the addition of NaF, desiccated thyroid, or iodine as the case might be. The treatment given is summarized as follows:

Period and ration additions

LOTS	NUMBER OF ANIMALS	PERIOD 1 (2 WEEKS)	PERIOD 2 (4 WEEKS)	PERIOD 3 (3 WEEKS)	PERIOD 4 (2–6 WEEKS)
I	3	Basal only	NaF	NaF	NaF plus D.T.
II	4	Basal only	Thyroidectomized	NaF	NaF plus D.T.
III	3	Basal only	Basal only	D.T.	D.T. plus NaF
IV	4	Basal only	Basal only	KI	KI plus NaF

Whenever NaF was added to the ration it was incorporated at the rate of 0.15 per cent. This level of ingestion is sufficiently toxic to allow continued growth in the young animal, but at a greatly retarded rate. In the mature animal it permits a fair degree of body weight maintenance, but is in itself not lethal for as long as six months' feeding. Desiccated thyroid[2] was fed at the rate of 0.25 per cent of the ration. This is one-fourth of the toxic level used by Loumous in 1934. When KI was used it was fed at the rate of 0.0328 per cent of the ration. Later this was increased to 1.64 per cent.

Basal metabolisms were determined in an apparatus patterned after that used by Forbes in 1934 but certain changes were made to facilitate greater accuracy and laboratory manipulation. The animals were placed in wire screen cages fitted with a removable tin bottom in which folded filter paper absorbed most of the excreta moisture.

The animal and cage were inserted in a glass container properly fitted for submerging in a constant temperature water bath at 28.2°C. Air was drawn through $CaCl_2$, soda lime, H_2SO_4, and ascarite into the respiration chamber. By means of an oil manometer the rate of air flow could be kept constant for each determination. Air flow was maintained at approximately 30 liters per hour. H_2O and CO_2 absorption was accomplished in 4 inch absorption tubes which could be weighed easily. The water was absorbed by dehydrite and the CO_2 by ascarite. The wire cage and fitted tin bottoms allowed the weighing of the animals outside of the glass submerging container. The animals were kept in the apparatus sufficiently long to lose 0.50 gram or more in body weight (weighed accurately to the second place) and to assure the production of an accurately measurable quantity of H_2O and CO_2. No determinations were

[2] The desiccated thyroid used in these studies was furnished through the kindness of the Wilson Laboratories and carried a guarantee of 0.35 per cent iodine.

TABLE 1

Weekly basal metabolism averaged by lots and periods

PERIOD I	LOT I—BASAL RATION				LOT II—BASAL RATION				LOT III—BASAL RATION			LOT IV—BASAL RATION			
	Weeks				Weeks				Weeks			Weeks			
	1	2	3	4	1	2	3	4	1	2	3	1	2	3	4
R.Q.	0.84				0.80				0.81			0.81			
Cal. per kgm. per hr.	5.73				5.94				5.72			6.22			
Cal. per sq. m. of body surface	827				811				851			979			
Period II	Basal ration with 0.15% NaF added				Thyroidectomy performed-Basal ration				Basal ration continued			Basal ration continued			
R.Q.	0.84	0.82	0.80	0.73	0.85	0.81	0.81	0.78		0.83	0.80		0.78	0.81	
Cal. per kgm. per hr.	5.25	5.03	5.02	5.91	4.15	3.79	4.84	5.60		4.44	4.27		5.82	7.63	
Cal. per sq. m. of body surface	815	770	794	886	636	562	709	844		720	712		880	1088	
Period III	0.15% NaF continued				Basal ration with 0.15% NaF added				Basal ration with 0.25% desiccated thyroid			Basal ration with 0.0328% KI added			
R.Q.	0.73	0.71			0.77	0.76	0.77		0.73	0.77	0.76	0.76	0.77	0.76	
Cal. per kgm. per hr.	3.90	5.98			5.78	5.01	5.89		4.53	5.22	7.21	5.43	4.31	4.95	
Cal. per sq. m. of body surface	617	825			884	777	872		736	883	1167	859	695	789	
Period IV	Ration of period III with 0.25% desiccated thyroid added				Ration of period III with 0.25% desiccated thyroid added				Ration of period III with 0.15% NaF added			Ration of period III with 0.15% NaF added			
R.Q.	0.73				0.76	0.75			0.75	0.78	0.74*	0.79	0.75	0.74	0.74
Cal. per kgm. per hr.	10.81				8.04	11.53			9.80	12.91	15.80*	4.64	5.41	4.27	5.80
Cal. per sq. m. of body surface	1425				1113	1559			1472	1589	2150*	719	765	653	826

* One animal only. It died 1 hour after this determination.

made for less than $1\frac{1}{4}$ hour period and in no case was a period longer than $2\frac{1}{2}$ hours necessary. With a little experience a fair degree of skill enables the operator to determine the basal metabolism of this species satisfactorily by the method just outlined.

All animals were starved from 24 to 48 hours before each determination. It was found impossible to starve the animals receiving NaF or desiccated

TABLE 2

Average daily ingestion of fluorine, iodine, and desiccated thyroid

PERIOD	LOT I	LOT II	LOT III	LOT IV
Daily fluorine intake in milligrams				
1	None	None	None	None
2	5.2	None	None	None
3	5.2	4.7	None	None
4	6.9	6.3	7.5	5.6
Daily fluorine intake in milligrams per kilogram of body weight				
1	None	None	None	None
2	27	None	None	None
3	27	24	None	None
4	27	44	37	25

PERIOD	D.T.	D.T. IODINE	D.T.	D.T. IODINE	D.T.	D.T. IODINE	D.T.	K.I. IODINE
Daily desiccated thyroid (D.T.) and iodine intake in milligrams								
1	None	None	None	None	None	None	None	None
2	None	None	None	None	None	None	None	None
3	None	None	17.3	0.060	28.5	0.100	None	3.2
4	25.5	0.089	23.2	0.081	27.6	0.097	None	5.6
Daily desiccated thyroid and iodine intake in milligrams per kilogram of body weight								
1	None	None	None	None	None	None	None	None
2	None	None	None	None	None	None	None	None
3	None	None	89	0.31	109	0.38	None	13.0
4	198	0.68	162	0.57	137	0.48	None	9.3

thyroid for more than 32 hours each week and preserve a fair degree of health. This interval was sufficient in view of the fact that their daily food intake was only 8 to 10 grams. The usual procedure was to remove the animals from access to feed 24 hours prior to the test. The tests were then made between the 24th to 32nd hour. In the case of the other animals, it was found necessary to starve them for 40 to 48 hours before placing them in the apparatus.

Results. During the first weeks of the experiment there was a tendency for the animals to gain, or maintain body weight, with the exception of lot I which ingested on the average 5.2 mgm. of F daily. This lot lost weight for three weeks until most of the body fat was depleted. Thereafter body weight was maintained. During this interval the daily fluorine ingestion was maintained at 5.2 mgm., or at the rate of 27 mgm. per kilo of body weight as shown in table 2. Desiccated thyroid introduced into the ration in addition to NaF during the fourth period caused a sharp and rapid decline in body weight which ended in collapse and death within

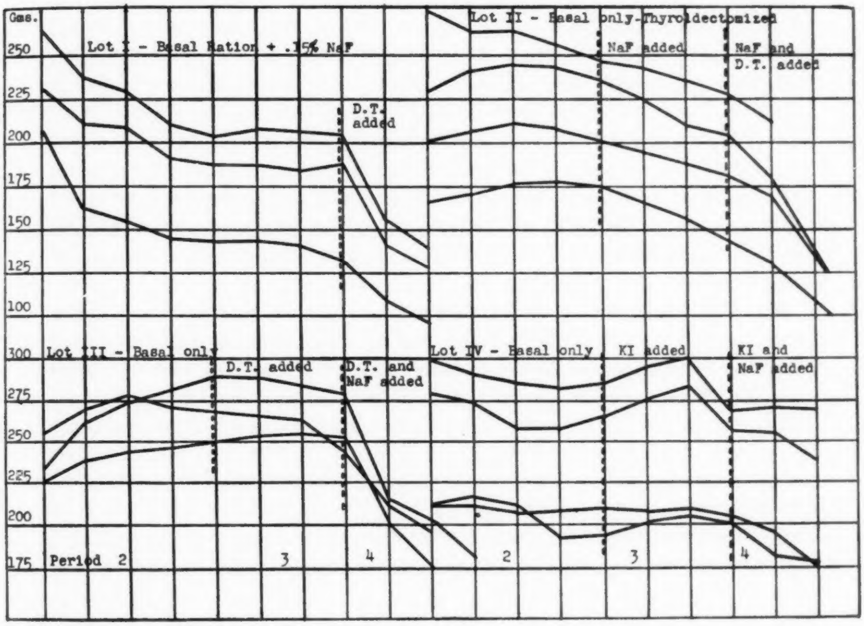

Fig. 1. Average body weight curves

two weeks. The average rate of desiccated thyroid ingestion of this lot was 25.5 mgm. daily, or 198 mgm. per kgm. of body weight. This was equivalent to an intake of 0.09 mgm. of iodine daily, or at the rate of 0.68 mgm. per kgm. of body weight. Desiccated thyroid alone in lot III produced no visible effects during the three weeks of period 3. This lot ingested an average of 27.6 mgm. of desiccated thyroid, or at the rate of 137 mgm. per kgm. of body weight in period 4. This represented an iodine intake of 0.1 mgm. daily. The addition of NaF to the diet of this lot during this period produced the same result as the combination of

fluorine toxicosis and desiccated thyroid in lot I. The disastrous loss of body weight was not produced by a combination of 0.0328 per cent KI and NaF. The iodine intake of lot IV was many times (16 to 42x) that fed in the form of desiccated thyroid. When the KI was increased to 1.635 per cent of the ration or 50 times the original level of iodine and fed with NaF no marked disastrous results were obtained with the animals tested at this level.

It is plainly evident from an inspection of figure 1 that 0.15 per cent NaF (0.068 per cent F) does not lead to a rapid and severe loss in body weight. Experience has shown us that this level of fluorine ingestion is tolerated for long periods by the rat. Likewise, 0.25 per cent desiccated thyroid has little effect upon body weight during short periods of time. Yet desiccated thyroid and NaF ingested together at the levels fed in this experiment enhances their toxicity to the point of a rapid and severe loss in body weight, which caused a fatal collapse within 14 to 19 days. A medium level of iodine, 0.025 per cent iodine in the form of KI, has no such pronounced effect. An iodine level of 1.25 per cent or 50 times the original level failed to produce the immediate toxic effects of thyroid when fed in combination with NaF, although it produced a decline in body weight.

The basal metabolism determinations were very satisfactory. Occasionally an animal became nervous and did not quiet down after being placed in the apparatus. In such cases the determination was made again at a later hour the same day or the data were omitted from the summary. For the most part the summary data presented in table 1 represent complete individual records averaged by lots for each week indicated. In period 4 lot I failed to survive the 24 hour starvation period in preparation for the 2nd basal metabolism determination. Lots II and III survived until the basal metabolism was determined the second week of period 4 with one exception in lot III which survived long enough for a determination the third week.

Examination of table 1 shows that the basal metabolic rate of the animals used in this experiment averaged very much the same before being subjected to the influence of NaF, desiccated thyroid, KI, thyroidectomy, or combinations of the same. The average basal metabolic rate for all animals before subjection to experimental dietary additions was found to be 861 calories per square meter of body surface, or 5.7 calories per kgm. per hour.

The addition of 0.15 per cent NaF to the ration of lot I failed to lower the basal metabolism in 7 weeks when the ingestion of fluorine was 27 mgm. per kgm. of body weight. Thyroidectomy in lot II showed a progressive reduction in the basal metabolic rate for two weeks. During the third and fourth week normal metabolism was again established.

Thereafter they behaved much as normal animals. These animals were kept on the experiment until they collapsed from NaF-desiccated thyroid toxicosis. Post-mortem examination showed that no regeneration of thyroid took place at the site of the operation. The addition of NaF to the ration of this lot caused no reduction of the basal metabolic rate.

The addition of 0.25 per cent desiccated thyroid to the ration of lot III caused a progressive rise in the basal metabolic rate which reached 1167 calories per square meter of body surface at the end of 3 weeks, or an increase of 35.5 per cent above normal.

During the fourth period when NaF and desiccated thyroid were fed simultaneously to lot I, the basal metabolic rate rose within one week to an average of 1425 calories per square meter of body surface or an increase of 65 per cent above normal. The rise in basal metabolic rate of lot II during period 4 was also distinctly more rapid than that produced by desiccated thyroid alone and increased approximately 80 per cent above normal at the end of the second week. The addition of NaF to the ration of lot III (desiccated thyroid fed lot) during the fourth period likewise occasioned a further rise in the metabolic rate from an average of 1167 calories to an average of 1531 calories per square meter of body surface. These results demonstrate that chronic levels of NaF enhance the toxicity of desiccated thyroid or vice versa. They show that NaF in the presence of the excess metabolic stimulants contained in desiccated thyroid not only does not reduce the stimulatory action of the material but quite to the contrary greatly augments the action. This finding may in part explain our observation that fluorine poisoned animals exhibit extreme emaciation.

The influence of iodine in doses of 6 to 9 mgm. of iodine (as KI) per kgm. of body weight caused a slight reduction of the basal metabolic rate in a 3 week period. The addition of 0.15 per cent NaF to the ration of lot IV (iodine fed lot) did not influence the effect of the iodine. Since the animals in this lot were in good health they were continued on the experiment. During the fourth week of period 4 one pair of animals was given NaF only while the remaining two were continued on the same level of KI and NaF. Beginning the 5th week KI was added to the ration of the NaF fed pair in sufficient quantity to increase the iodine intake approximately 50 times or at the rate of 1.64 per cent KI. Following the temporary reduction of the metabolic rate caused by the iodine administration the normal rate was again established. No distinct rise in metabolic rate was observed to accompany iodine feeding. High iodine feeding resulted in a noticeable loss of body weight.

Discussion. The notion that fluorine toxicosis produced cretinism led Goldenberg to undertake his study on the action of fluorides in the control of thyrotoxicosis in humans. In our experiments the animals

fed fluorine showed marked loss of body weight, depletion of fat stores, anorexia and inanition. The animals were active until the effects of inanition curtailed much of the voluntary muscular activity. These results confirm those obtained with other species which have been used for the study of chronic fluorine poisoning in this laboratory, i.e., cattle, swine, guinea pigs, and chickens. This is quite distinctly different from the characteristically lowered basal metabolism of the cretin who nearly always exhibits obesity, permanently retarded growth, more or less myxoedema, and sluggish mentality. These results as well as former experience indicate to us that the syndrome of chronic fluorine poisoning is distinctly different from that of cretinism.

The interaction of fluorine and desiccated thyroid presents a distinct and definite picture of enhanced toxicity. The manner in which it is produced is as yet speculative. It is possible in view of the recent evidence of Collip and co-workers (1935) to visualize the inactivation of the antithyroid hormones, or the enzymatic systems involved in their elaboration by chronic fluorine poisoning. It appears that a more active substance than thyroxine, such as a fluorine-substituted thyroxine in which fluorine replaces the iodine of the molecule wholly or in part, may be responsible for the increased toxicity observed.

The fluoride effects have been to increase the action of the metabolic stimulants found in desiccated thyroid. While no evidence is available at present, it is possible that fluorine may be an etiological factor in the syndrome of "hyperthyroidism" in susceptible persons, since fluorine has been found to be present in fairly large quantities in many drinking waters.

SUMMARY AND CONCLUSIONS

A daily intake of 27 mgm. of fluorine per kilogram of body weight (rats) produced no disastrous effect upon body weight. Neither did the ingestion of 198 mgm. of desiccated thyroid effect body weight in a short period of 3 weeks. A combination of the two produced a marked effect upon body weight which resulted in collapse and death in two to three weeks. A combination of KI at a level of 0.038 per cent and NaF at 0.15 per cent of the ration failed to produce a disastrous exhaustion of body substance.

A normal basal metabolic rate was established for the rats used in this series of experiments at 5.7 calories per kgm. per hour, or 861 calories per square meter of body surface per 24 hours. The addition of NaF at the levels fed in these experiments failed to lower, or cause any appreciable change in the normal basal metabolic rate of the rat.

The feeding of 0.25 per cent desiccated thyroid increased the basal metabolic rate of the rat approximately 35 per cent in 21 days. The addition of 0.15 per cent NaF to the ration containing 0.25 per cent thyroid

caused a sharp rise in the basal metabolic rate. A gain of 65 per cent was noted within 7 days. In all cases the combination of NaF and desiccated thyroid caused a sharp and rapid rise of the basal metabolic rate. Iodine alone failed to elicit this response. On the contrary the addition of 0.0328 per cent of KI to the ration caused a temporary reduction in the metabolic rate. NaF in combination with KI did not change the metabolic rate other than that noted by iodine alone.

These results lead inevitably to the conclusions: that fluorine in the form of NaF does not lower the basal metabolic rate of the normal rat; that NaF augments and therefore enhances the toxicity of "hyperthyroidism" induced by feeding desiccated thyroid; that insofar as the spontaneous "hyperthyroidism" of toxic goiter and "hyperthyroidism" induced by the administration of desiccated thyroid are identical, to that extent NaF therapy is contraindicated; and that a ration containing a combination of 0.15 per cent NaF and 0.25 per cent desiccated thyroid rapidly produces an exhaustion of body weight and a fatal collapse while either substance alone has no marked effect, and is not fatal.

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FETAL CARBOHYDRATE METABOLISM FOLLOWING ADRENALECTOMY, INSULIN AND GLUCOSE EXPERIMENTS ON THE MOTHER

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Changes in fetal carbohydrate levels following operations on the maternal organism have been previously reported in the literature by a number of investigators (Carlson and Drennan, 1911; Naeslund, 1928; Pack and Barber, 1928, 1930; Schlossman, 1930; Britton, 1930; Corey, 1932; Stewart and Higgins, 1935, and others). Reviews of the rather voluminous literature have been published by Needham (1931, 1933). The majority of reports on the subject have been limited to a single phase of the problem, and a rather disconcerting lack of agreement is apparent concerning many points. Confinement of study to a single mammalian form and the establishment of an adequate series of normal values before attacking the experimental phases of the problem appeared advisable.

Following a study of the growth rates of the fetal liver and placenta of the rat and the determination of normal glycogen levels in the maternal liver, fetal liver and placenta during the latter third of the gestation period (Corey, 1935), it was considered feasible to investigate the possible effects on fetal carbohydrate economy of various experimental conditions imposed on the mother. The procedures dealt with in the present report include *a*, adrenalectomy; *b*, the administration of cortico-adrenal extract; *c*, the induction of insulin hypoglycemia, and *d*, the injection of glucose solution. The normal series of animals referred to above served as controls, with the addition of several rats fasted for different periods and others from which the spleen and one adrenal gland had been removed. A total of over 600 fetuses comprising 89 litters was used in these experiments. The method employed in making the glycogen determinations (a modified Pflüger technique) is referred to in the earlier report cited above. The results of all experiments have been summarized in table 1 (see below).

Effects of adrenalectomy. The tendency of pregnant rats to abort rendered it difficult to obtain litters in which the mother had been operated on for prolonged periods of time prior to sacrifice. This tendency to abortion in adrenalectomized animals has already been commented on

(Britton and Kline, 1934), and the present observations are confirmatory: thus out of 31 operated animals, only 19 litters were retained *in utero* for a sufficiently extended period to allow their use in the experiments described.

Fetuses removed from adrenalectomized animals an average of 5 days after operation showed a markedly depleted liver glycogen storage. No significant changes were noted in the fetal liver weight or total solid content, and the placental and fetal muscle glycogen appeared, moreover, to be but little affected. Tabulation of the results obtained indicated, furthermore, that the reduction in fetal liver glycogen concentration might be correlated with the degree of adrenal insufficiency experienced by the mother:

	NUMBER OF LITTERS	MATERNAL LIVER GLYCOGEN	FETAL LIVER GLYCOGEN
		grams per cent	*grams per cent*
Controls: Groups (5), (6), (7)	36	2.18	4.86
Adrenalectomized 3–4 days	10	0.81	3.13
Adrenalectomized 5–8 days	9	0.61	2.45

Whether the reduction in fetal liver glycogen following adrenalectomy in the mother represents a disturbed fetal carbohydrate metabolism due to a diminished supply of cortico-adrenal hormone from the mother, or merely reflects the maternal hypoglycemia brought about by adrenalectomy, must remain for the present a matter of conjecture. In view of the findings of Naeslund, Schlossman, Snyder and Hoskins (1928) and Stewart and Higgins, which indicate a fairly free transmission of glucose through the placenta, the latter view may perhaps be considered the more tenable.

Cortico-adrenal extract treatment. Daily intraperitoneal injections of cortico-adrenal extract (3 cc. per day) for an average period of 5 days failed to bring about significant alterations in either the maternal or fetal glycogen values. The extract used was made in this laboratory according to a modified Swingle-Pfiffner technique employed by Britton and Silvette (1931) in experiments on functional aspects of the adrenal cortex.

It appeared that an over-abundance of cortical hormone in the maternal circulation had no effect on fetal carbohydrate metabolism. No conclusions could therefore be drawn from this study regarding the placental permeability of the extract used.

Maternal hypoglycemia after insulin. All (10) animals received one unit of insulin intraperitoneally per 100 grams of body weight, and were sacrificed at the end of 6 hours for excision of tissue samples. Eight of the 10 rats employed gave evidence of insulin shock. The maternal

blood sugar fell from an average of 135 to 53 milligrams per cent during the experimental period.

The similarity of the results following adrenalectomy and insulin injection (see table 1) tends to support the contentions of Britton and Silvette (1932) concerning the rôle of the adrenal cortex in carbohydrate metabolism.

No alteration in weight of the fetal liver was detected following insulin injection in the mother; maternal and fetal hepatic glycogen was, however, considerably reduced. Failure of the fetal muscle glycogen to show a decreased concentration in the present experiments requires further elucidation. It is perhaps noteworthy that the placental glycogen remained unaffected, in agreement with the contentions of Huggett (1929).

TABLE 1

EXPERIMENTAL PROCEDURE	NUMBER OF LITTERS	AVERAGE CROWN-RUMP LENGTH	AVERAGE WEIGHT OF FETUSES	FETAL LIVER			GLYCOGEN			
				Average wet weight	Per cent of body weight	Average solid content	Liver		Placental	Fetal muscle
							Maternal	Fetal		
		mm.	grams	grams	grams	grams	grams per cent	grams per cent	grams per cent	grams per cent
(1) Adrenalectomized. Sacrificed (Av.) 5 days later	19	35	3.3	0.225	6.7	0.048	0.70	2.80	0.35	0.51
(2) Cortico-adrenal extract; 3 cc. daily for (Av.) 5 days	15	35	3.9	0.224	6.0	0.047	1.53	4.80	0.41	0.50
(3) Insulin; 1 unit per 100 gms. Killed after 6 hrs.	10	34	3.3	0.232	6.8	0.044	0.69	2.94	0.35	0.48
(4) Glucose (2%) 5 cc. twice daily for 2 days	9	35	3.7	0.275	7.5	0.059	4.28	7.20	0.30	0.72
(5) Controls; fasted 12 hrs.	30	31	2.9	0.235	8.6	0.046	2.31	4.86	0.50	0.40
(6) Controls; fasted 24 hrs.	4	35	3.7	0.219	6.2	0.047	1.72	4.96	0.40	0.40
(7) Controls; spleen and 1 adrenal removed, 3 days	2	34	3.3	0.223	6.6	0.047	1.22	4.77	0.30	0.42

Glucose injection. Intraperitoneal injection in the mother of 2 per cent glucose solution (5 cc. twice daily) over a period of 2 days produced no change in the weight of the fetal liver, although a slight increase in the solid content of that organ was indicated. The maternal glycogen level rose to 4.28 compared with an average of 2.18 grams per cent for the 3

control groups. An increase in the fetal liver glycogen to 7.20 per cent compared with an average of 4.86 for the control groups indicated a definite increase in glycogenic concentration. This observation does not agree with the findings of Lochhead and Cramer (1907) who reported that "neither the placental store of glycogen nor that of the fetal liver is affected by feeding the animals on a diet rich in carbohydrates." Stewart and Higgins, on the other hand, found that the fetal liver glycogen (rat) fluctuated with that of the mother after feeding, and the present experiments appear to confirm their observations.

The fact that the placental glycogen value was not markedly altered agrees with the observations of Huggett, who found that the percentage of glycogen in the placenta (rabbit) was not altered by starvation, carbohydrate feeding, injections of carbohydrates, or administration of adrenalin and thyroxin.

Control experiments. These cases included fasting the mothers for 12 and 24 hours, and the removal of the spleen and one adrenal gland in two instances. Although the maternal liver glycogen level in the animals fasted for 24 hours was somewhat lower than that observed in those following a 12-hour fast, the fetal glycogen level was not found to be significantly disturbed. Moreover, the wet weight and total solid content of the fetal liver were found to be similar in both groups. The fact that the percentage of the fetal body weight represented by the liver, as well as the placental glycogen level, were higher in the 12-hour fasted group, may be explained by the fact that in this series the fetuses were on the average younger than in the second and smaller group. Thus, it has been observed in this laboratory that the liver is relatively large in the young rat fetus and that the placental glycogen content falls from the earlier stages until term. A similar condition as regards the placental glycogen has been observed in the rabbit by Lochhead and Cramer.

Removal of the maternal spleen and left adrenal gland had no significant effect on the fetal liver weight or glycogen values. The possibility that the slight depression which was observed in the glycogenic levels might be attributable to mild adrenal insufficiency produced by the unilateral operation cannot, however, be overlooked.

SUMMARY

Materno-fetal carbohydrate metabolism in the rat was studied by determining the glycogen concentrations in the maternal and fetal livers, the placenta and fetal muscle of normal animals, and after subjection of the mother to various experimental procedures.

Adrenalectomy of the mother produced significant depletions in the glycogen content of the maternal and fetal livers: in 19 cases there was an average reduction of the hepatic glycogen of 70 per cent in the mother and 43 per cent in the fetus, compared to 12-hour fasted controls.

Injections of cortico-adrenal extract in pregnant rats failed to evoke alterations in the various carbohydrate values studied.

The administration of insulin to the mother was followed by decreases in maternal and fetal liver glycogen similar to those observed after adrenalectomy.

Injections of glucose into the mother resulted in an increase in glycogen concentration in the fetal liver, as well as in that of the mother.

The placental glycogen remained relatively unaffected by the different experimental procedures employed, and the fetal muscle glycogen exhibited no significant change except a slight rise following glucose injection in the mother.

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AN HISTAMINE-LIKE SUBSTANCE IN THE GASTRIC JUICE

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Histamine has been found in several organs of the ox, including the intestinal mucosa (1), the lung and liver (2), the heart (3) and the spleen (4). Also it was isolated by Abel and Kubota (5) in 1919 from the gastric and intestinal mucosa of the dog, and by Sacks, Ivy, Burgess and Vandolah (6) in 1931 from the pyloric mucosa of the hog. Best and McHenry (7) in studies on the inactivation of histamine reported varying concentrations in several tissues of the dog, indicating a wide distribution in the body. The largest amounts per kilogram of tissue were found in the liver, the lungs and gastro-intestinal tract. Essentially similar concentrations were found in the stomach, the duodenum, the jejunum and the cecum. The presence of histamine in so many fresh normal tissues suggested that it might be of some physiological importance; yet quantitative determinations, indicating considerable amounts in the body, pointed to a very efficient means of either elimination or inactivation. Best and McHenry (7) in 1930 discovered an histamine-inactivating substance for which they suggested the name histaminase. In the dog, histaminase was found in the kidney, intestinal tract, blood, muscles, spleen, lung, adrenals, bladder and liver. The intestines and kidneys were found to be the richest sources of the inactivating substance, while none was found in the stomach, heart or skin. It is interesting that the stomach, the secretory activity of which is greatly increased by injection of histamine subcutaneously, contains an appreciable amount of histamine but no histaminase.

Although histamine has been isolated from the gastric mucosa, there is hitherto no direct evidence of its presence in gastric juice. Positive results on the stimulation of gastric secretion by the injection of gastric juice into dogs might suggest its presence or that of some similar substance (8, 9). However, negative results have also been obtained in this regard (10, 11). This study is an investigation of whether or not histamine is secreted by the stomach. If such were the case it might be

possible to demonstrate its presence in the gastric secretion, inasmuch as there would be no histaminase present to inactivate it.[1]

METHODS. *Collection of gastric juice.* Human gastric juice, undiluted and devoid of food, was obtained by continuous aspiration. The juice present in the fasting stomach was removed, kept as a separate specimen, and referred to as the "fasting" sample. Then histamine acid phosphate, in the dose of 0.1 mgm. per 10 kgm. of body weight, was injected subcutaneously and the subsequent gastric secretion was collected for from two to three hours as a single specimen, and referred to as the "after histamine" sample. In order to accumulate large quantities for extraction

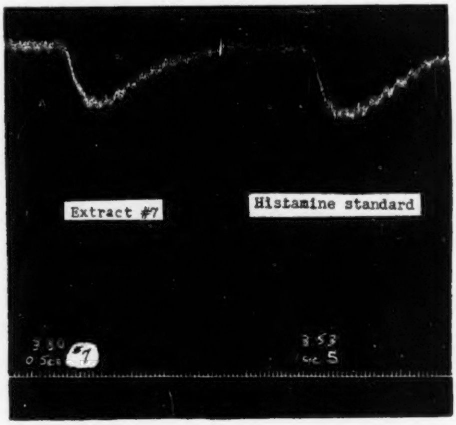

Fig. 1. Assay by the blood pressure method.

Kymographic record of the evanescent lowering of the blood pressure (in the etherized, vagotomized cat) produced by the standard histamine solution (graph on right) and comparison with the change produced by the gastric juice extract (graph on left). Sample 7: original volume of gastric juice 200 cc.; final extract volume 10 cc. Standard histamine solution: 1 cc. = 0.004 mgm. histamine acid phosphate. Assay: 0.0140 mgm. per 100 cc. gastric juice, calculated as histamine base.

purposes, specimens of juice from several aspirations were pooled together, the juice being kept at 4°C. until starting the extraction procedure.

Extraction and assay. The method of extraction of histamine from tissues used by Best and McHenry (7) was applied to gastric juice. The volumes of the samples submitted to the process ranged from 100 cc. to 10 liters, most of them being from 500 cc. to 1000 cc. The final extracts from these samples ranged in volume from 5 cc. to 30 cc., and were water clear or slightly yellow in color.

[1] This work in part was reported (by title) at the Twenty-fourth Annual Meeting of the American Society for Clinical Investigation, held in Atlantic City, N. J., May 2, 1932 (12).

Extraction of the smaller samples was started almost immediately after aspiration of the juice was completed. The accumulation of the larger samples required considerable time before extraction could be done, ranging from a few days to a few weeks, the juice, as mentioned above, being kept at 4°C. Although it seemed unlikely, it was not known whether the

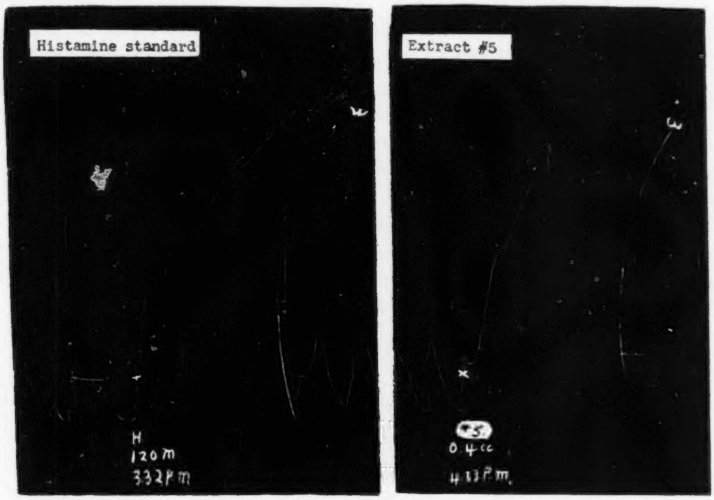

Fig. 2. Assay by the guinea pig uterine strip method.

Kymographic record of the violent contraction of the strip of virgin guinea pig uterus produced by the standard histamine solution (graph on left) and comparison with the change produced on the same uterine strip by gastric juice extract, sample 5 (graph on right). The normal rhythmical contractions are seen in the fore part of each graph. The test solutions were brought into contact with the uterine strip on the "up stroke" of the contraction as indicated by a cross mark. The point of removal of the test solution and washing of the uterine strip is indicated by the letter W seen near the top of the graph, to be followed immediately by a "down stroke" and subsequently by reëstablishment of the normal rhythmical contractions. Volume of tissue bath, 100 cc. Standard histamine solution: 1 to 20 million dilution; made up of 0.005 mgm. histamine acid phosphate in 100 cc. Ringer's solution. Extract 5: Original volume of gastric juice, 200 cc.; final volume of extract, 10 cc. Test solution of extract 5 made up of 0.4 cc. of extract in 100 cc. Ringer's solution. Assay: 0.0225 mgm. per 100 cc. gastric juice, calculated as histamine base. (Assay of extract 5 by blood pressure method, 0.0309 mgm.)

gastric juice kept under these conditions for such a time would undergo any destruction or production of histamine as a result of bacterial or chemical reaction. So control observations were made over the longest time any sample was kept, and these indicated no appreciable change in the potency of the histamine-like activity in the gastric juice.

The extracts were assayed by comparison of their physiological activity with that of a standard solution of histamine on the blood pressure of the etherized, vagotomized cat. Doses of histamine differing from each other by 15 per cent may be discriminated by this method (13). A sufficient number of controls after atropinization of the test animal were done to rule out a possible blood pressure lowering effect from choline compounds. An example of the comparable curves is given in figure 1, showing the evanescent lowering of the blood pressure produced by gastric juice extract, sample 7, similar to that produced by the standard histamine solution.

Although not used generally in this study as an assay method, a few of the gastric juice extracts were tested for their effect on the virgin guinea pig uterine strip. The kymographic record of such a test, together with the histamine control, is illustrated in figure 2.

TABLE 1

Extracts of gastric juices from normal young adults

Assay values of histamine-like activity calculated as milligrams histamine base per 100 cc. juice.

SAMPLE NUMBER	ASSAY CALCULATED AS MGM. HISTAMINE BASE PER 100 CC. GASTRIC JUICE	REMARKS
1	Not potent	"Fasting" juice
3	0.0013	"Fasting" juice
6	0.0160	"Fasting" juice
9	0.0535	"Fasting" juice
2	0.0026	After histamine
4	0.0023	After histamine
5	0.0309	After histamine
7	0.0140	After histamine
8	0.0165	After histamine
10	0.0411	After histamine

Best and McHenry (7) used their extraction method on amounts of tissue as small as 20 grams; and they stated that "added histamine can invariably be recovered from tissues with an error of not more than 15 per cent." If histamine were present in the gastric juice it would seem probable that the amount would be very small, or at least in very weak dilution. So the applicability of this method to gastric juice and the efficiency of recovery of such small amounts of histamine were uncertain. Therefore, control samples containing known minute amounts of histamine, as histamine acid phosphate, in 100 cc. of distilled water were extracted and assayed by the standard procedure. Ten samples containing the equivalents of from 0.00036 mgm. to 0.018 mgm. of histamine base were assayed as "not potent" after extraction. Three further samples containing 0.036, 0.072 and 0.180 mgm. were assayed, after extraction, to contain 0.0006,

0.018 and 0.068 mgm. respectively, expressed as histamine base. A specimen of gastric juice was divided into two 100 cc. samples. The first one, after extraction, was assayed to contain 0.0047 mgm. of histamine. To the second was added histamine acid phosphate (the equivalent of 0.036 mgm. of histamine base). When extracted, this sample was assayed to contain 0.025 mgm. of histamine. Such results indicate that the extraction method is usable when samples contain only minute quantities of histamine, but that there is no recovery if the content is less than 0.018 mgm. calculated as histamine base.

DATA. *Extracts of juices from normal young adults.* The assay values of ten samples of gastric juice from normal young adults are given in table 1. None of these specimens were stained with bile, and in procuring them, great precaution was taken to avoid contamination with saliva. Samples 1 (not potent, "fasting") and 2 (0.0026 mgm., "after histamine") were obtained from the same individual on the same day; samples 3 (0.0013, "fasting") and 4 (0.0023 mgm., "after histamine") were obtained from a second individual on the same day. The assay values of these samples indicate a higher histamine activity after stimulation by histamine. On the other hand, the average of the three potent "fasting" samples (0.0236 mgm.) as compared with an average of the six "after histamine" samples (0.0179 mgm.) indicates a higher histamine activity in the "fasting" samples. These averages, however, are not subject to reliable interpretation because some of the samples (nos. 6, 8, 9 and 10) represent accumulated specimens from several aspirations and several individuals, to provide larger quantities of juice for extraction.

TABLE 2

Extracts of gastric juices from patients with peptic ulcer

Assay values of histamine-like activity calculated as milligrams histamine base per 100 cc. juice.

SAMPLE NUMBER	ASSAY CALCULATED AS MGM. HISTAMINE BASE PER 100 CC. GASTRIC JUICE	LOCATION OF ULCER	REMARKS
11	Not potent	Stomach	Fasting
12	0.0036	Stomach	After histamine
13	0.0031	Stomach	After histamine
14	0.0062	Duodenum	After histamine
28	0.0047	Duodenum	After histamine

Extracts of juices from patients with peptic ulcer. Assay values of gastric juice extracts from four patients with untreated peptic ulcer are given in table 2. Samples 11 ("fasting") and 12 ("after histamine") were obtained from the same patient on the same day. None of these cases had gastric retention and the gastric juice contained no gross bile.

Extracts of gastric juice from a miscellaneous group of patients. Thirteen samples of gastric juices from a number of patients with functional gastric disturbances in whom x-ray examination revealed no organic gastrointestinal lesion, were extracted and assayed. All samples contained free hydrochloric acid. These samples, in order to provide large quantities of juice for extraction, were comprised of pooled specimens from several aspirations and several individuals. Therefore they represent no one individual nor any one disease. The values obtained were 1, for fasting samples: 0.0091, 0.0105, 0.0224 and 0.0203; 2, for samples after histamine administration: 0.0240, 0.0191, 0.0029, 0.0165, 0.0206, 0.0071, 0.0074, 0.0064 and 0.0053 expressed as milligram of histamine base per 100 cc. of gastric juice. The main purpose of this part of the investigation was

TABLE 3

Assay values with reference to presence and absence of protein constituents in the gastric juice

SAMPLE NUMBER*	ASSAY CALCULATED AS MGM. HISTAMINE BASE PER 100 CC. GASTRIC JUICE	
	Protein present (control)	Protein precipitated out by alcohol
18 (19)	0.0091	0.0105
21 (20)	0.0203	0.0224
23 (22)	0.0206	0.0165
25 (24)	0.0074	0.0071
27 (26)	0.0053	0.0064
Average................	0.0125	0.0125

* The number given first in the column belongs to the assay value of the sample containing protein, while the number in parenthesis belongs to the assay value of the companion sample from which the protein was precipitated by alcohol.

to further evaluate the methods of extraction when large quantities (as much as 10 liters) of juice were available, and in general, to further establish the presence or absence of histamine in the gastric juice, rather than to obtain assay values to correlate with those from normals.

One sample comprised of gastric juice from several cases of pernicious anemia was found to have an assay value of 0.0166 mgm. per 100 cc., calculated as histamine base. Because of the difficulty in obtaining this juice it was necessary to dilute the specimens by instillation of small amounts of distilled water into the stomach. Therefore, this observation is of no value except to indicate the presence of an histamine-like substance in the gastric juice in pernicious anemia.

Do the protein constituents in the gastric juice influence the assay value when the histamine extraction method of Best and McHenry is employed?

In the beginning of this investigation it was not known definitely whether the acid digestion procedure of Best and McHenry could form histamine from the protein constituents of the gastric juices. Five samples, each divided into two equal portions while the juice was still fresh, were used to study this question. One portion served as a control, while the second portion was treated with alcohol to precipitate the protein and filtered. Of the second portion only the filtrate was submitted to the extraction procedure. The assay values of these extracts are given in table 3. It is apparent that no histamine was produced by the extraction process. This result conforms with the control studies on the procedure reported by Gavin, McHenry and Wilson (14) in which they carried egg albumin, pure casein and solutions of histidine hydrochloride through the method and could demonstrate no depressor action in the resultant extracts.

Attempts to isolate histamine chemically from gastric juice. Five unsuccessful attempts were made to isolate histamine as the dipicrate from gastric juice known to be potent by assay methods. The quantities of juice submitted to analysis in the individual trials were respectively 2 liters, 2 liters, 5 liters, 10 liters and 10 liters.

COMMENT. The presence of an histamine-like substance in the gastric juice has been demonstrated by biological methods. In twenty-eight samples only two were found to be "not potent," and both of these were juices obtained from the fasting stomach. These samples represented gastric juices from normal young adults, patients with peptic ulcer, and a miscellaneous group of patients with functional gastric disturbances. Although this study provides quantitative data obtained by approximate assay, the inability of the present methods to recover efficiently very small amounts of histamine makes it impossible to estimate accurately the total original content in the gastric juice. The controls, as reported above, indicate that one could not expect to recover in the final extract more than from one-sixth to one-third of the substance originally present in the gastric juice. Therefore, at this time any conclusion as to the normal quantity of this histamine-like substance in the gastric juice or its variation in disease is not warranted. It has been demonstrated in the juice obtained from the fasting stomach as well as in that secreted after histamine stimulation. Some of the observations suggest that this substance is present in larger quantity after histamine stimulation. This is of interest in view of the studies of Gavin, McHenry and Wilson (14) in which they found the histamine content of canine gastric tissue, both pyloric and fundic, was greater after a meat meal than when fasting. If gastric secretion after histamine stimulation were considered comparable to that produced by food, the assay values of the juice obtained "after histamine" would be the most significant in attempting to arrive at a figure for the normal content of gastric secretion. The average assay

value for the six "after histamine" samples from normal young adults (see table 1) is 0.0179 mgm. Judging from the results of our control extractions, it is estimated that the average amount of histamine-like substance normally present in gastric juice is about 0.7 mgm. per liter, with a very wide range from less than 0.3 mgm. to less than 1.8 mgm. per liter.

Absolute identification of histamine demands that it be isolated chemically. Our inability to do this, while disappointing, is not entirely unexpected when one considers that a large proportion may be lost in the chemical procedures. Taking the average figure estimated above, the largest quantity, 10 liters, submitted to chemical isolation possibly would contain only about 7 mgm. Other work with tissue (4) has indicated that only about 10 per cent recovery of histamine may be expected by the chemical isolation procedure. Best and McHenry (15) consider "the inference that histamine is present is legitimate if the results of a complete physiological analysis of a solution are identical to those obtained with pure histamine." The effects of the gastric juice extracts on the cat's blood pressure (see fig. 1) and on the guinea pig uterus (see fig. 2) apparently are identical with those produced by pure histamine solution, and the inference that the histamine-like substance in the gastric juice actually is histamine seems justified.

In the light of present knowledge the significance of histamine in the gastric secretion is unknown. The concentration of 0.7 mgm. per liter could not indicate any important means of elimination, nor could it account for any appreciable effect as a gastric secretory hormone through reabsorption. Most workers have found that large doses of histamine, when given by mouth, are required to stimulate gastric secretion. Lim, Ivy and McCarthy (16) found, in the dog, that 50 mgm. histamine acid phosphate in 20 cc. solution was the minimum quantity, applied locally to the gastric mucosa, which would cause gastric stimulation. In man, as much as 100 mgm. to 225 mgm. (17) of histamine by mouth may be required to stimulate gastric secretion. However, Fenyes (18) reported increased gastric secretion from much smaller doses by mouth (2 mgm. to 12 mgm.).

Demonstration of histamine in blood suggests the possibility that histamine in the gastric juice may represent transudation through the mucosa. It would be of interest to know if histamine were a constituent of the secretions from other segments of the alimentary tract. It appears impossible practically to determine if it is secreted by the intestine, since there is a large amount of histaminase in intestinal tissue, and also bacterial production of histamine may occur in the intestinal tract. W. Kamenowa (19) demonstrated a histamine-like substance in human saliva. Best and McHenry (7) found dog's blood to contain 0.4 mgm. histamine per kilogram, but the presence also of histaminase in the blood

makes it difficult to evaluate this figure. However, the concentration of histamine in gastric tissue (in the dog) is so much greater than that in blood that the theory of transudation seems untenable.

CONCLUSION

An histamine-like substance has been found to be a constituent of human gastric juice.

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THE EFFECT OF VIOSTEROL UPON OXYGEN CONSUMPTION OF FROG'S MUSCLE[1]

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Since viosterol in large doses has been found to increase the metabolic ratė in dogs (Reed, Thacker, Dillman and Welch, 1933), and in rats (Reed, 1934), and even in moderately excessive doses may stimulate the metabolic rate in children (Hess, Poncher, Dale and Klein, 1930), the question naturally arises whether an increase in respiration can be demonstrated in the isolated muscle under the influence of viosterol. A failure to demonstrate an increase of respiration in the isolated muscle from a viosterolized animal would indicate that the influence is lost as soon as the muscle is no longer exposed to viosterol in the blood. If that were true, one would expect that the addition of viosterol to the muscle *in vitro* should increase the respiration. On the other hand, if the increased oxygen consumption is maintained after excision of a muscle from the animal previously treated with viosterol, it would indicate that the latter had modified the cell activity sufficiently to maintain a higher metabolic level for some time after the tissue is no longer in contact with the viosterol.

The experiments here reported were undertaken to determine the oxygen consumption of isolated muscle from animals that had been given viosterol.

METHODS. From each batch of frogs half of the total number selected at random were given daily injections (peritoneally) of 0.1 cc. of irradiated ergosterol in the form of 10,000X viosterol, which contains 920,000 International Units per cubic centimeter. The other half were used as controls. For each experiment the sartorius muscle was dissected out and left in Ringer's solution for two hours. It was then placed in a Thunberg-Fenn respirometer and the oxygen consumption was measured in the usual manner. Readings were taken every 10 minutes and values were used for calculation of the oxygen consumption when the rate became constant. All experiments were rejected when a constant rate was not established. The temperature of the bath in which the respirometer was immersed was 25°C. for all experiments.

[1] The expenses of this work were defrayed in part by the Wisconsin Alumni Research Foundation, and the viosterol was generously furnished by Mead Johnson and Company.

RESULTS. In table 1 are given the results on two groups of frogs. The experiments on group I were carried out between December 19 and February 4, 1934, and on group II between April 4 and May 3, 1935. The oxygen consumption not only varies from one frog to another, but also from one batch of frogs to another. The latter, and also the seasonal difference, are most likely responsible to some degree for the difference of the average oxygen consumption between the two groups of frogs. It will also be noticed that the average weight of the sartorius muscles in the first group is somewhat less than in the second, which would also account for some of the difference in respiration, since a greater diffusion of gases would be expected from the smaller muscles.

In both group I and group II the average rate of oxygen consumption of those frogs that have been given viosterol is greater than that of the controls. In the first group it is 29.8 per cent, and in the second group 18 per cent greater. In figures 1 and 2 are plotted the oxygen consumption rates of groups I and II respectively, showing the difference of respiration levels between the injected and controls and also the extent of variation among all of the frogs. In group I the difference in the respiratory levels between the injected and controls is more pronounced than in group II, although the degree of variation in the latter group is much smaller.

TABLE 1

	GROUP			
	I		II	
	Control	Injected	Control	Injected
Number of experiments	9	9	10	10
Mean oxygen consumption in cubic millimeters per gram fresh weight per hour	51.5	71.5	34.2	40.4
Mean weight in milligrams	70.0	65.6	97.7	90.8
Mean number of injections		5.6		4.2
Per cent difference in O_2 consumption	29.8		18	

A statistical analysis of the data, using Fisher's (1928) equations in testing the significance of difference of means of small samples, yields a figure for P between 0.05 and 0.02 for group I. This indicates that the probability of the observed differences between the treated and controls being due to chance sampling, is between 1 chance in 20 and 1 chance in 50. This can be considered significant. For group II, however, P is slightly greater than 0.1, which is not statistically significant. Upon examination of figure 2 it will be noticed that one point in each of the injected and control series is very much out of the range of the rest of

the values, which are quite uniform for oxygen consumption readings. If one, then, omits these two values from the statistical treatment one obtains a value for P which is in the range of the one obtained for group II.

Though the effect of the viosterol on the respiratory rate of the muscle is not striking, it does, nevertheless, appear significant. On the other hand, it is hardly to be expected that a much greater increase in the oxygen consumption rate would result from the viosterol administration. It might also be pointed out that the maximum number of injections administered to any one frog was 8, the average number for both groups being slightly under 5.

The increased respiration of muscle from viosterol treated frogs is analogous to the increased respiration of tissue from thyroid treated animals (Hopping, 1931; Hicks, 1932; Gerard and McIntyre, 1932). If the action of viosterol in raising the metabolism is similar to that of thyroxin, one would not expect any effect from the addition of viosterol to isolated tissue *in vitro*. It is rather difficult, however, to test this point since

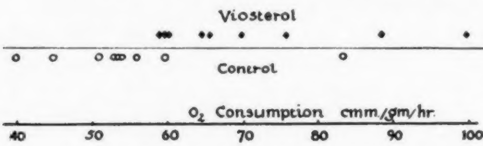

Fig. 1

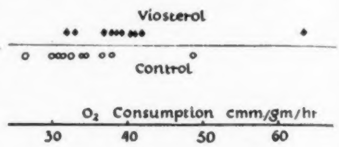

Fig. 2

viosterol is not soluble in water and is obtained only in oil solution. It is quite possible, furthermore, that the viosterol in some way stimulates the thyroid. The metabolic effect would, consequently, be due to the latter mechanism. This possibility is being experimentally investigated.

SUMMARY

Isolated muscles from frogs receiving daily injections of viosterol have a greater oxygen consumption than the controls.

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BLOOD REGENERATION IN SEVERE ANEMIA[1]

Fractions of Kidney, Spleen and Heart Compared with Standard Liver Fractions

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Fractions obtained from liver and known to be potent in human as well as experimental anemia hold an interest for physicians, laboratory workers and chemists alike. A great deal of work has been done in this country as well as abroad in an attempt to isolate, purify and eventually to identify and synthesize one or more of these potent factors. Progress has been made but these substances are elusive and although obtained in apparently high concentration have not yet been classified. Dakin and West very recently have reported important findings (2).

From time to time we have published observations (5) dealing with *liver fractions* tested on our standard anemic dogs. We have tested a liver fraction (4) known to be potent in *human pernicious anemia* and found that this fraction contained 10 to 20 per cent the potency of whole liver as standardized in experimental secondary anemia. Another fraction of liver was shown (11) to contain about 75 per cent the potency of whole liver as tested in *experimental secondary anemia* (table 4 below) and this fraction has been used with success in the treatment of certain human secondary anemias.

With this background, it seemed desirable to prepare and test similar fractions from spleen, kidney and heart muscle. If such fractions showed unusual distribution of potency as compared with liver fractions a lead might be uncovered to indicate the nature of these substances. This hope was not realized but the evidence obtained from these new fractions gives support to the view that not one but *several factors* are contained in the viscera which have a potent influence upon the regeneration of red cells and hemoglobin in experimental anemia.

Methods. General routine procedures and methods used in the stand-

[1] We are indebted to the Rochester Packing Company for a liberal supply of certain meat products.

[2] Mr. Walden is a member of the Lilly Research Laboratories.

ard experimental anemia due to blood withdrawal in dogs have been described in detail (6, 10). The *fractions* tested below are prepared as follows: Fresh tissue is finely ground into water containing sufficient sulphuric acid so that the pH of the final mixture is between 5 and 6. This mixture is then heated to 80 or 85°C., held at this temperature for about one hour and filtered. The filtrate is evaporated to a thick syrup in a vacuum at low temperature. To this is added sufficient alcohol to render the final concentration approximately 70 per cent by volume. The portion insoluble in 70 per cent alcohol is dried to a powder and designated the *secondary anemia fraction.* The portion soluble in 70 per cent alcohol is again concentrated to a thick syrup and added to a large volume of alcohol to produce an alcoholic concentration of 92 to 95 per cent by volume. This portion which is soluble in 70 per cent alcohol and insoluble in high alcohol is dried in vacuum and powdered and designated as the *pernicious anemia fraction* (1). The *residue* is the portion of the gland which is insoluble in the hot acid water used as the initial extracting medium, and is dried and powdered. The preparation of these fractions was done in the Research Laboratories of Eli Lilly & Company.

Net hemoglobin production above the control basal level has been given particular attention during the last two years and the figures used in all tables below are *net figures.* All anemic dogs have been fed for 10 to 12 weeks or longer on the standard salmon bread and the basal output for each dog determined as the stated grams hemoglobin per week. This standardization is repeated once or twice a year to be certain that no change has developed. The fraction or residue is then added to the basal ration for two weeks and the total output determined for these two weeks and the following two weeks on the standard bread. From this total hemoglobin output is deducted the basal output for four weeks giving the *net hemoglobin output* for the given fraction or residue. The hemoglobin level in the blood by means of blood withdrawal is kept as constant as possible usually within 5 per cent of the anemia level of 45 per cent (100 per cent = 13.8 gm. hemoglobin). If the anemia level at the start is 47 per cent hemoglobin and at the end of the four week test period is 43 per cent, we deduct 4 grams hemoglobin from the total output to adjust the level to the original 47 per cent. In these dogs a change of 1 per cent hemoglobin reading is equivalent to a little more than 1 gram hemoglobin in circulation at this anemia level.

Experimental observations. It will be noted in the tables below that the sum of the hemoglobin output coming from the separate fractions is greater than the total hemoglobin output coming from the whole tissue fed. This is particularly noticeable in table 3 where the beef heart residue has approximately the same potency as the whole tissue. The sum of the two fractions and residue is more than twice the output noted for the

whole fresh heart tissue. This is frequently observed in fractionating organ tissue for other potent factors. We believe the method of testing these fractions is reasonably accurate and the average figures therefore should have some significance. It may be argued that the method of preparation may free substances which are held unavailable in the protein matrix of the fresh tissue; or different combinations of amino acids and other factors coming into the body may influence the hemoglobin metabolism in different ways.

Table 1 gives a series of carefully conducted experiments on the same dogs using various fractions of pig *kidney* tissue. A number of new points can be made from the figures given in table 1. The potency for pig kidney is not as close to liver as the earlier reported experiments indicated. With liver as 100 grams hemoglobin per 2 weeks feeding, we see that kidney

TABLE 1

Kidney—pig

Hemoglobin production in grams per 2-week feeding period—300 grams daily intake or fraction derived from 300 grams.

DOG NO.	SECONDARY ANEMIA FRACTION NO. 55		PERNICIOUS ANEMIA FRACTION NO. 343		RESIDUE		FRESH TISSUE	
	Kidney fraction	Liver control	Kidney fraction	Liver control	Kiduey residue	Liver control	Kidney	Liver control
30-116	58	90	48	90	50	90	84	90
27-236	42	94			15	112	59	92
30-114	28	94	39	99	25	94	65	99
26-18	43	121	53	121	49	121	77	121
							83	
27-235							76	100
Average...	43	100	47	103	35	104	74	100

rates only 74 grams. *Kidney residue* also is low in potency as compared with the residues of liver, spleen and beef heart. The secondary anemia fraction contains slightly less potency than the pernicious anemia fraction and the ratio averages 43 to 47 grams hemoglobin per 2 weeks feeding. This is conspicuously different from the liver where this same ratio of comparison reads 75 to 15 (table 4). No adequate explanation is at hand.

Table 2 gives figures for hemoglobin regeneration in anemia due to fractions of *pig spleen* added to the basal diet. In our early reports on spleen (8) we used *calves' spleen* which was not as potent and found 20 to 50 grams hemoglobin production due to feeding 150 to 300 grams calves' spleen daily for 2 weeks. Table 2 shows that *pig spleen* is more potent and feeding 300 grams daily will result in 87 grams hemoglobin production

per 2 weeks. Figures for iron are given in table 5. The secondary and primary anemia spleen fractions are a little less potent than those derived from the kidney. The spleen residue is rich in potent material in spite

TABLE 2

Spleen—pig

Hemoglobin production in grams per 2-week feeding period—300 grams daily intake or fraction derived from 30 grams.

DOG NO.	SECONDARY ANEMIA FRACTION NO. 55		PERNICIOUS ANEMIA FRACTION NO. 343		RESIDUE		FRESH TISSUE	
	Spleen fraction	Liver control	Spleen fraction	Liver control	Spleen residue	Liver control	Spleen	Liver control
27-240	43	93	43	93	93	93		
26-102	23	100	22	100				
27-236	48	94	41	92				
23-1	43	99	31	93				
29-67					44	100	69	93
30-115					66	118		
30-121					56	98		
32-2							100	100
32-5							91	104
Average...	39	97	36	95	65	103	87	99

TABLE 3

Heart muscle—beef

Hemoglobin production in grams per 2-week feeding period—300 grams daily intake or fraction derived from 300 grams.

DOG NO.	SECONDARY ANEMIA FRACTION NO. 55		PERNICIOUS ANEMIA FRACTION NO. 343		RESIDUE		FRESH TISSUE	
	Heart fraction	Liver control	Heart fraction	Liver control	Heart residue	Liver control	Heart	Liver control
27-240	47	93	33	95	66	93	54	98
27-234	39	100	32	100	36	100	47	102
27-234							49	109
27-235	25	100	32	100	62	100	55	90
27-241	49	118	34	128	67	118	62	118
30-114							49	89
Average...	40	103	33	106	58	103	53	101

of its small volume. The potency is 65 grams hemoglobin output per 2 weeks on an intake of spleen residue which represents only 4.3 per cent the fresh weight of spleen. The spleen residue is as potent as the liver residue but weighs only $\frac{1}{5}$ as much. Both residues are rich in iron but the liver residue per 300 grams fresh equivalent is three times as rich in iron as the spleen.

Table 3 gives the net figures for hemoglobin regeneration in anemia due to fractions of *beef heart* added to the basal diet. Beef heart obviously contains about one-half the potency of pig liver but a point of interest is that the beef heart *residue* after the chemical treatment outlined in the method contains as many potent factors as the whole fresh beef heart. This fact has been discussed above.

The secondary and pernicious anemia fractions derived from beef heart deserve further study as they contain minimal amounts of iron and in spite of considerable potent materials weigh only about 1 per cent of the

TABLE 4

Liver—pig

Hemoglobin production in grams per 2-week feeding period—300 grams daily intake or fraction derived from 300 grams.

DOG NO.	SECONDARY ANEMIA FRACTION NO. 55		PERNICIOUS ANEMIA FRACTION NO. 343		RESIDUE	
	Liver fraction	Liver control	Liver fraction	Liver control	Liver residue	Liver control
29-65	68	101				
27-235	99	106				
29-66	52	103				
27-240	73	92				
27-240	71	92				
27-240	77	92				
29-67	62	104				
27-234	95	109			72	104
27-241	72	87			51	87
27-241	82	118			71	101
27-241	69	87	25	87	39	87
26-102					82	100
24-25			16	91		
24-26			15	104		
24-45			0	99		
25-97			20	103		
Average..	75	99	15	97	63	96

fresh weight. In other words in 1 per cent of the original weight with little or no iron to confuse the study we have a potency of 33 to 40 per cent that of whole liver. For example a dog which receives 46 grams of the secondary anemia fraction per 2 weeks (3.3×14) will produce 40 grams new hemoglobin—almost a one to one ratio of intake to output. There is only a very small amount of iron concerned (see table 5).

Table 4 gives the net figures for hemoglobin regeneration in anemia due to fractions of pig *liver* added to the basal diet. The "secondary anemia fraction" is particularly carefully standardized and shows that the original

figures given (11) are correct—that this fraction in 3 per cent of the fresh liver weight contains 75 per cent the potency of whole liver. The "pernicious anemia fraction" gives figures of 15 per cent potency for hemoglobin regeneration in secondary experimental anemia although it contains full potency for therapy of human pernicious anemia. The *residue* as in the case of the spleen retains a large amount of potent material and gives potency figures of 63 per cent of whole liver.

It is to be recalled that in these diet experiments with the anemic dogs we feed daily for 2 weeks 300 grams of fresh tissue or the fraction derived

TABLE 5

Average hemoglobin output compared with iron intake derived from various fractions and organ tissues

	KIDNEY, PIG	SPLEEN, PIG	HEART MUSCLE, BEEF	LIVER, PIG
Secondary anemia fraction no. 55:				
Average hemoglobin output per 2 weeks, gm	43.0	39.0	40.0	75.0
Iron—daily intake, mgm	6.5	14.7	2.2	26.4
Weight ratio of fraction to fresh tissue, per cent	1.4	1.2	1.1	3.0
Pernicious anemia fraction no. 343:				
Average hemoglobin output per 2 weeks, gm	47.0	36.0	33.0	15.0
Iron—daily intake, mgm	2.5	1.3	0.2	0.6
Weight ratio of fraction to fresh tissue, per cent	2.5	3.5	1.1	4.3
Residue:				
Average hemoglobin output per 2 weeks, gm	35.0	65.0	58.0	63.0
Iron—daily intake, mgm	12.6	13.2	27.1	30.4
Weight ratio of fraction to fresh tissue, per cent	16.7	4.3	20.0	22.0
Fresh organ tissue:				
Average hemoglobin output per 2 weeks, gm	74.0	87.0	53.0	100.0
Iron—daily intake, mgm	24.5	55.5	15.3	54.2

from 300 grams of fresh tissue. Contained in this fresh tissue or the derived fractions is a variable amount of *iron* and the figures given in table 5 indicate the milligrams of iron contained in 300 grams of organ tissue or fractions derived from 300 grams of fresh tissue which is the daily intake. As might be expected the residue contains the largest amount of this tissue iron and the pernicious anemia fraction (no. 343) contains least. It is at once apparent that the fresh tissue does not always contain the same amount of iron as the sum of the iron in the derived fractions from the same type of organ tissue (table 5). The fresh tissue was obtained from local sources while the fractions were prepared from material obtained in the middle West.

The basal ration also contains *iron* (20 mgm. iron per 300 grams salmon bread, the customary daily ration) and the iron added to this basal ration by the various fractions is usually insignificant. In the case of the spleen more than half of this iron is contained in blood which is held in the spleen sinusoids and it is well known (9) that the dog can utilize only about 10 to 15 per cent of ingested hemoglobin to build new hemoglobin in anemia.

The liver is the main storehouse for the body iron and the fractions are in harmony but there is striking contrast between the secondary and pernicious anemia liver fractions. The spleen may contain as much or more iron per unit weight than the liver but in total bulk it contains only a small fraction of that found in the liver. The kidney contains only small amounts of iron. The heart muscle contains a good deal of iron and about half is almost certainly contained in the muscle hemoglobin molecule. The very low figures for iron in the pernicious anemia fraction of beef heart are of interest (table 5).

The weight of the various fractions and residues are also given in table 5. The pernicious anemia fractions are a little heavier as a rule. The spleen residue is surprisingly small and makes up only 4.3 per cent of the fresh weight as compared with approximately 20 per cent for the kidney, beef heart or liver.

Discussion. It will be noted that the *hemoglobin production* due to *pig liver* feeding is remarkably uniform in these standard anemic dogs and the average net figure is 100 grams hemoglobin per 2 weeks. This figure of course is the net figure after deduction of the individual basal output due to the standard salmon bread. This net figure is also in excess of the maintenance factor which is an unknown figure and accounts for the daily wear and tear and wastage of red cells in the body which obviously must be made up from the total blood output. This daily maintenance factor in the dog may amount to 1 or 2 per cent of the total circulating red cells and hemoglobin (3).

This wear and tear percentage may be greater in anemia but this is pure speculation. One might argue that because of frequent bleedings and rapid new blood formation the red cells in circulation would be in large measure *new* and therefore have a long circulatory existence before them and a low wear and tear index. On the contrary one could argue that the anemia conditions might put more strain on the relatively few red cells in circulation, therefore shorten the life expectancy and increase the wear and tear or wastage index. Actually the red cells from the anemic dogs are more resistant to hemolysis by hypotonic solutions than are red cells from non-anemic dogs.

In other publications (4) we have recorded the *hemoglobin index*—the ratio of hemoglobin in per cent divided by the red cell hematocrit in per

cent. This hemoglobin index is not recorded in the many experiments reported in brief in this paper but this index was studied carefully with the hope that some of these fractions might modify this index which should give information as to the saturation of the red cell matrix by the contained hemoglobin. These fractions do not change appreciably the hemoglobin index in these experiments but we have observed an increase in this index in many experiments where large amounts of liver or iron or both were given over two or more weeks. This might indicate increased saturation of the red cell matrix (stroma) with hemoglobin but the reverse of this we have been unable to produce regularly in these standardized anemic dogs.

We have tested from time to time purified liver fractions suitable for intramuscular injection and known to be potent in human pernicious anemia. This material does not give any unusual response in these anemic dogs but the reactions are not uniform and will not be reported at this time. A Ventriculin-like preparation likewise has been tested with results not unlike those reported (7) for fresh beef stomach but these experiments will not be reported here.

SUMMARY

Various fractions of liver have been tested on standardized anemic dogs. The potent substance for *pernicious anemia* therapy is largely contained in a fraction which contains but little of the potent factors effective in *secondary* anemia due to blood loss in dogs. Potent factors for secondary anemia are concentrated in a separate fraction. These two fractions of liver are contrasted in table 4 above.

Similar fractions prepared by the same methods from pig spleen, pig kidney and beef heart are standardized in experiments tabulated above. Some interesting differences in potency are to be noted.

It is very easy to dissociate the therapeutic effect of iron from the potency of these various fractions and residues.

All evidence points to *several factors* rather than a single factor as responsible for the potent influence of these fractions upon the regeneration of red cells and hemoglobin in experimental anemia due to blood withdrawal.

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THE ACTION POTENTIALS OF THE AUDITORY NERVE[1]

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Action potentials in the auditory nerve and tracts of the midbrain were first detected by Buytendijk (1910). They were subsequently studied by Forbes, Miller and O'Connor (1927). In 1930, Wever and Bray described the striking correlation between the frequency of the electrical disturbance led off from the eighth nerve and the sound waves used as stimuli. The correspondence, which is good enough to enable spoken words to be recognized clearly, has become known as the Wever and Bray phenomenon. It now appears that this effect depends upon a mixture of action potentials of the nerve fibers and another electrical effect. The latter is apparently generated by the cochlea and reproduces closely the wave form of the stimulating sounds (cf. Adrian, 1931: Adrian, Bronk and Phillips, 1931; Davis, Derbyshire, Lurie and Saul, 1934). The nature of this "cochlear response" is still somewhat in doubt. Hallpike and Rawdon-Smith (1934b) apparently favor a neural theory of its origin, while we (Davis, Derbyshire, Lurie and Saul, 1934, and Lurie, Davis and Derbyshire, 1934) have presented evidence pointing to the sensory cells of the organ of Corti as its source. We have now extended our studies of the auditory mechanism to the action potentials in the auditory nerve and their relation to the cochlear response and, as far as possible, to certain features of hearing.

Method. The apparatus used is that described by Garceau and Davis (1934). It consists essentially of a resistance-capacity coupled amplifier and cathode ray oscillograph. For auditory stimulation a dynatron oscillator generates electrical sine waves of 60 to 12,000 c.p.s., which after amplification are sent to either one of two loud-speakers. The "low" speaker covers the range from 30 to 3500 c.p.s. The "high" speaker covers the range from 3000 to 15,000 c.p.s. The loud-speakers are connected by short pieces of rubber hose to a sliding brass coupling. When

[1] The experimental work here described was performed in partial fulfillment by one of us (A.J.D.) of the requirements for the degree of Doctor of Philosophy at Harvard University. The apparatus employed was originally constructed by E. L. Garceau, the expenses being defrayed by grants from the DeLamar Mobile Research Fund of the Harvard Medical School, from the Josiah Macy, Jr. Foundation, from the American Otological Society and from other anonymous donors.

either speaker is coupled to the main sound tube, the diameter of the sound tube is uniform throughout. Beyond the coupling is a 17-meter length of hose with a short brass coupling 6 meters from the sliding joint. This second brass coupling has a side tube of 6 mm. internal diameter that fits snugly into the speculum which is sewed into the cat's ear. The usual distance along this tube and the cat's auditory canal to the ear drum is about 55 mm. Opposite the side tube is another hole of 6 mm. diameter leading directly into a brass chamber that accommodates the condenser microphone.

A thyratron oscillator generating sharp electrical impulses may be connected to the same loud-speakers to deliver clicks at 60 per second.

In these experiments cats are used under moderate avertin anesthesia. The usual dose is 240 mgm. of avertin fluid per kilo of body weight, injected intraperitoneally. The bulla and round window are exposed as described by Davis, Derbyshire, Lurie and Saul (1934), and, in addition, the squamous bone is removed to the limits of the tentorium and the petrous bone. The dura is split and enough of the cerebellum is scooped out to allow a clear view of the eighth nerve. It is preferable that the horn of the plexus of the fourth ventricle, which overlies the nerve, be left intact to avoid trauma from further dissection.

Coaxial needle electrodes of the type described by Adrian and Bronk (1929) are used for recording from the auditory nerve. These electrodes are firmly held in micromanipulators so that small movements are easily and precisely made. The electrode system for recording from the round window comprises a wick soaked in Ringer's solution applied to the membrane of the round window, and a grounded silver plate on the neck muscles.

To distinguish between the action potentials and the cochlear response, two routine tests are employed. First, the latency of the responses to clicks shows that wherever detected there is no more than 0.1 ms. delay between the arrival of a sound wave at the ear drum and the appearance of the cochlear response. The action potentials are always delayed at least 0.5 ms. after the cochlear response. Secondly, by reversing the electrical polarity of the thyratron output, the polarity of the sound wave reverses, so that what was a positive pressure becomes a negative pressure. In response to this procedure, the cochlear phenomenon reverses its polarity like the sound waves. The action potentials, on the other hand, never do so.

These tests indicate that when stimulated by low tones the records from the round window comprise roughly 25 per cent action potentials and 75 per cent cochlear response, while the electrodes in the auditory nerve detect only action potentials. Examples of these tests will be given below.

Results. *Equilibration.* A preliminary description of these phenom-

ena was published by Davis, Forbes and Derbyshire (1933) and also by Davis (1934). Continuous photographic records are taken of the response of the auditory nerve evoked by tones at a strength of stimulation of about 60 or 70 db above threshold. This response is maximal, i.e., it shows no further increase with increase in intensity of the stimulus. From continuous records on films (fig. 1) the amplitude of the second excursion and also the amplitude after 2 seconds of stimulation are measured. The first response is neglected, because it is essentially part of an "on effect" (Davis, Derbyshire, Lurie and Saul, 1934), but, because this effect is rapidly damped out, the second wave is chosen for measurement. The relation that this "initial response" bears to the frequency of stimulation is shown in figure 2. The amplitude remains constant until a critical frequency is reached, usually about 900 c.p.s., and then it falls to a little less than one-half. It remains at this level until an octave higher, at which frequency (*ca.* 1800 c.p.s.) it falls again. This time a level is established at less than one-third of the amplitude for low tones. The response to higher tones is too small to measure satisfactorily, and between 3000 and 4000 c.p.s. the synchronization of the action potentials with the stimulating frequency is entirely lost.

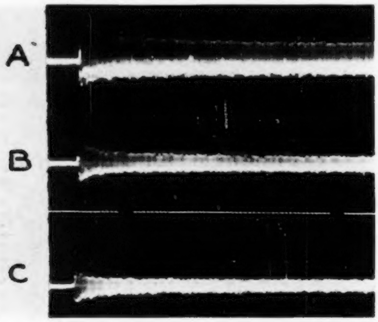


Fig. 1. (Expt. of Nov. 29, 1933.) Continuous oscillographic records showing equilibration, with and without alternation. Slightly retouched.

A. 310 c.p.s. No alternation. Initial height after "on-effect" 15 mm. on original film. Moderate equilibration.

B. 570 c.p.s. Partial alternation. Initial height 11 mm. Marked equilibration.

C. 875 c.p.s. Complete alternation. Initial height 8 mm. Moderate equilibration.

This experiment showed an unusually low critical frequency at which alternation appeared and a high degree of equilibration.

During the first 7 minutes following the onset of a stimulating tone, the action potentials shrink in size. The reduction is much greater and more rapid during the first 2 seconds of stimulation than during any later period, so that we may refer to this early phase as "fast" equilibration. The degree of "fast" equilibration is calculated as the percentage decrease from the response during the first 2 seconds of stimulation. For frequencies below 400 c.p.s. the reduction, thus measured, is slight. As the frequency increases, the reduction becomes more rapid and greater in degree, until at the critical frequency mentioned above, usually 900 to 1000 c.p.s., the degree of equilibration abruptly decreases. Above this critical frequency

the amplitude of the initial response is approximately one-half of that found below it. As the second critical frequency near 2000 c.p.s. is approached and passed, the same changes in the degree of "fast" equilibration occur again in the same order (see fig. 2).

Both the fall in initial response and the increased percentage of reduction of the action potentials repeat again near 3000 c.p.s. The size of the responses is so small, however, that while the shrinkage can be seen by inspection it cannot be satisfactorily measured.

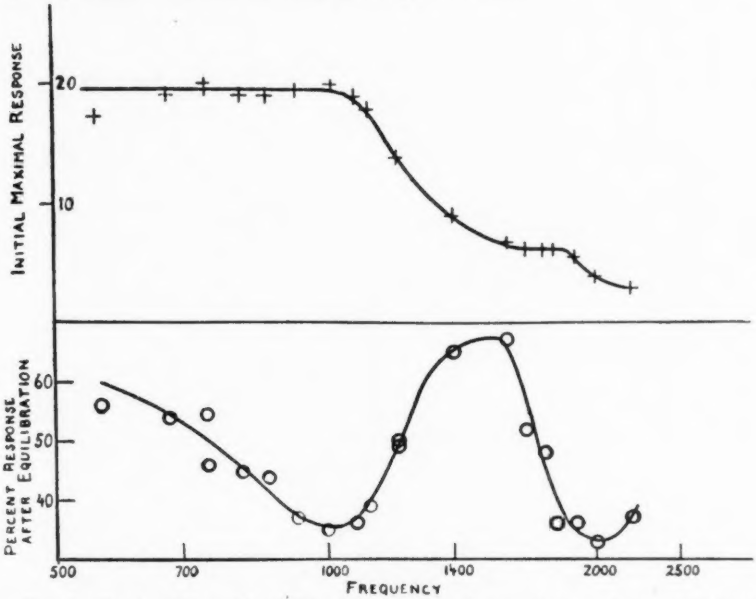


Fig. 2. (Expt. of Nov. 23, 1933.) Upper curve shows relation of size of "initial" response of the auditory nerve, measured after the "on-effect," to frequency. The critical frequencies of 1000 and 1900 are the highest in our series. The curve is unusual in the gradual fall above 1000 c.p.s.

Lower curve shows the percentage of this initial response remaining after 2 seconds' equilibration.

Our interpretation of these changes is based on the studies of fatigue in peripheral nerve by Forbes and Rice in 1929 and also by Gerard and Marshall in 1933. The diagram in figure 3 illustrates our explanation. The shrinkage of the response produced by 2 seconds of maximal stimulation is closely related to the number of impulses per second in each nerve fiber. At low frequencies, below 600 c.p.s., the reduction is not more than 30 per cent. However, at about 1000 c.p.s., the reduction is maximal and we infer therefore that this critical frequency evokes the greatest possible

number of impulses per second in each fiber. Because the degree of "fast" equilibration is the same at two frequencies, such as 1600 and 800 c.p.s., which are in a 2-to-1 ratio, one above and the other below the first critical frequency, we infer that the number of impulses per second in each nerve fiber is the same at both frequencies of stimulation. This fact, in conjunction with the observation that the initial amplitude at frequencies above the critical frequency (*ca.* 1000 c.p.s.) is nearly one-half that below the critical frequency, leads us to believe that the individual nerve fibers are responding only to alternate sound waves. Following the terminol-

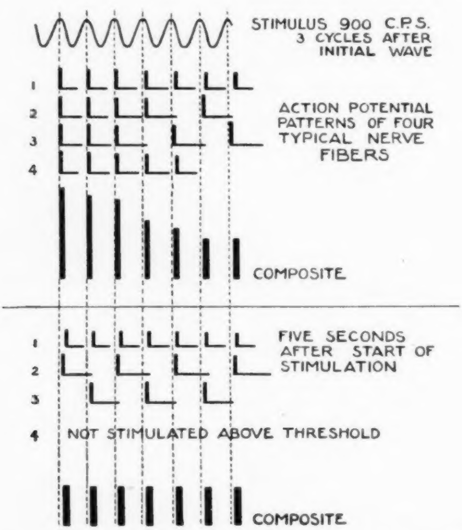


Fig. 3. Diagram of activity of 4 typical fibers of the auditory nerve. Vertical lines indicate the axon potentials, and the horizontal lines, the duration of the "functional" refractory period. "Composite" shows the sums of the axon potentials. The slight thickening of this line as stimulation proceeds indicates a slight temporal dispersion of these potentials.

ogy of Forbes and Rice (1929), we shall refer to this situation as "alternation" of response.

At frequencies above the second fall (*ca.* 2000 c.p.s.), each nerve fiber is responding only to every third stimulus, and the corresponding amplitude of the initial response is about one-third of the original. Evidence for a 4-to-1 ratio at still higher frequencies may occasionally be observed in fresh preparations.

The reduction in size of response that occurs during the first 2 seconds of stimulation is a combination of at least two phenomena. The action

potential of each fiber is decreasing in amplitude, as shown by Forbes and Rice (1929), and there is also an increase in the number of fibers which are forced to respond alternately as stimulation proceeds. These are both expressions of "equilibration" of the nerve as described by Gerard and Marshall (1933). The equilibration that occurs in 2 seconds and is predominantly due to alternation we call "fast" equilibration as opposed to the "slow" type to be discussed later. The "fast" equilibration depends primarily on the prolongation of the relatively refractory period described by Field and Brücke (1926).

Another phenomenon appears simultaneously with these changes in size and is predictable on the basis of the above interpretation of the diminution in the initial response. As the frequency is raised above, say, 250 c.p.s., each nerve impulse is set up closer to the relatively refractory period which follows the preceding impulse. If the second impulse is actually traveling in the relatively refractory period of the first, its velocity of conduction is slower than normal. Hence we observe that, at frequencies near 1000 c.p.s., immediately after the tone begins the impulses shift from left to right on the tube face of the cathode ray oscillograph. This means an increase in latency. The shift disappears near 1500 c.p.s., presumably as the result of an alternation of activity such that each fiber responds to alternate sound waves, and no impulse travels in the relatively refractory period of the preceding impulse.

The explanation of these phenomena offers an indirect determination of the recovery period of the auditory nerve. The freshest and best preparations show the first fall in initial amplitude near 1000 c.p.s. and the second fall near 2000 c.p.s. The "functional" refractory period for these fibers is therefore about 1.0 ms. We define the "functional" refractory period of a tissue as the shortest refractory period determined by stimulation through natural channels. It will usually be longer than the absolutely refractory period determined by direct artificial stimuli such as induction shocks. The functional refractory period depends in part upon the strength of stimulation of which the activating mechanism, in this case the sensory end-organ, is capable. It may also be determined by the rate of recovery of the slowest portion of the conducting pathway which the impulses must traverse.

Since the functional refractory period of every portion of the conducting path can be no longer than that of the system as a whole, it is evident in this case that the non-medullated peripheral terminations of the auditory nerve lying on the basilar membrane must possess a functional refractory period not longer than 1.0 ms.

The amplitude of the action potentials at the end of 2 seconds of stimulation is only a relatively constant value. If photographs are taken at appropriate intervals during 10 minutes following the first 2 seconds of

stimulation, the amplitude shows the further reduction illustrated in figure 4. The true steady state of the size of response is attained in 7 to 10 minutes after the onset of the stimulus.

The approximate time course of recovery following equilibration has been examined by keeping the film moving after the stimulus has been turned off and photographing the responses to brief test stimuli. Each period of stimulation is not longer than one-quarter of a second. The second response is measured in each sample. Figure 4 shows the resulting recovery curve. The original amplitude is attained in about 30 seconds, and for at least 1 minute thereafter the response is usually slightly greater than the original amplitude before fatigue.

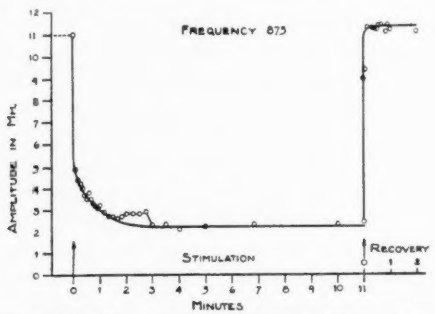


Fig. 4. (Expt. of Oct. 23, 1934.) Action potential of the auditory nerve in relation to duration of stimulation at 875 c.p.s., showing "slow" equilibration and recovery. The deviation of points from the smooth curve between 2 and 3 minutes does not appear in other experiments.

Stimulation which causes slow equilibration at any one frequency also reduces the response to tones of somewhat higher or lower frequencies, but the range of this effect has not yet been investigated.

During equilibration a certain number of fibers will drop out of action. Presumably, these fibers lie near the ends of the vibrating section of the basilar membrane, where the stimulus is only a little above threshold. As the threshold rises with equilibration, these fibers soon cease to respond. However, the number of such fibers (no. 4 in fig. 3) is probably small.

Sensitivity. The threshold of the electrical responses from either the round window or the eighth nerve is defined as the intensity of the tone yielding the first visible response on the cathode ray oscillograph. We find that audible thresholds, determined by listening for the responses from the loud-speaker, agree within a few decibels with the visible thresholds. Therefore, the audible thresholds of the asynchronous responses in the eighth nerve are comparable with the visible thresholds for the synchronized responses.

In order that a signal may be visible, it must rise above the "background." The "background" is due to amplifier noise, electrode noise, and the activity of the tissues near the electrodes. The amplifier noise contributes not more than 1 or 2 microvolts (μv.) to the "background." The electrode noise in the nerve may contribute from 2 to 4 μv., but the records from the round window usually show no disturbances greater than those of the amplifier. Occasionally, there is a greater noise-level at the round window, but we have not determined its cause in these special cases.[2] The activity of the tissues is probably due to injury from introducing the electrodes, but part of this activity has a functional significance as shown by the fact that it is lost by raising or lowering the electrodes.

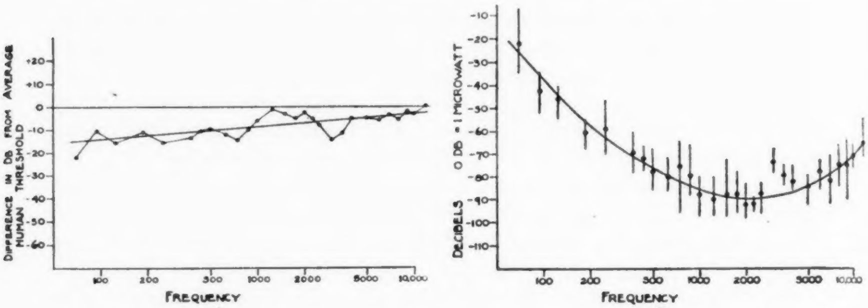
Thresholds of the responses at the round window are taken to compare with those from the auditory nerve. The threshold is measured as the decibels of attenuation of 1 volt (root mean square), delivered to the stimulating loud-speaker, necessary to produce the first visible response. The voltage (r.m.s.) sent to the loud-speakers is not strictly a measure of the sound pressure at the cat's ear drum because of the resonances and losses in the sound system; nor does this value take account of the frequency characteristic of the amplifiers. The method for dealing with these distortions is developed below.

A calibrated condenser microphone determines the pressures in its sound field. However, it is technically impossible to place the microphone in a position corresponding to the cat's external meatus without changing the acoustic impedance of the system from that which exists when the cat's ear terminates the side tube. Consequently, the difference between the sound pressures when the microphone is present and when the cat's ear is present is not measured. The microphone in its usual position opposite the side tube leading to the cat's ear is of little value in determining the pressures at the ear drum, but it does give a picture of the wave form of the stimulus.

On the assumption that the acoustic impedance of the human ear is similar to that of the cat's ear, a reference series of 8 normal human audibility curves was determined with the same acoustic system as for the cat. The average curve, when compared with a similar curve calibrated in absolute units by Sivian and White (1933) shows approximately the losses characteristic of our particular system of delivering the sound to the cat's or human ear. Approximate absolute values for the cat's threshold are obtained by adding to the crude values the differences in decibels between our normal human curve and that of Sivian and White.

[2] Observations on guinea pigs by one of us (H.D.) in collaboration with Dr. S. S. Stevens indicate that this increased noise-level is probably due to the action potentials of the tensor tympani muscle. If the anesthesia is light, this muscle remains in a state of tonic contraction.

These corrections do not account for the transmission losses in the amplifiers at different frequencies, because the human thresholds are obtained by a report from the observer and not by a response through the amplifiers. These amplifier losses are determined by calibration with electrical sine waves. Because the corrections apply to pure sine waves, only the responses from the round window, which are nearly sinusoidal, are subject to them.[3] The action-potential spikes are all of approximately the same



A B

Fig. 5. A. Average thresholds from 8 round windows of normal cats plotted as differences in decibels from the average thresholds of 8 human ears (all under 30 yrs. of age). The lower a point, the less the sensitivity, as in a standard audiogram.

B. Same data as in A, plotted in relation to 1 microwatt of sound energy at the ear drum. In this graph, the lower a point, the greater is the sensitivity represented. The data have been corrected for transmission losses and for amplifier attenuation as described in text.

The vertical bars indicate the extreme range of values at each frequency, not the standard deviation. The heavy dots show the averages of the values in decibels.

The great variability at 250, 750, and 875 c.p.s. is due in part to the simultaneous presence of both action potentials and cochlear response in phase relations which tend to reduce rather than increase the total wave. At 750 and 875 there is also some evidence that harmonics appear very readily in some cases, possibly dependent on the degree of contraction of the tensor tympani muscle (cf. footnote 2). The high points from 3000 to 4000 c.p.s. probably depend upon differences between resonant properties of the external auditory canal of the cat and of man.

shape irrespective of their frequency and therefore should all be corrected by the same amount. Consequently, no corrections for amplifier loss are applied to the thresholds or sizes of response of the action potentials.

[3] Because the threshold response is a small but finite value and because with weak stimuli the logarithm of the size of response changes linearly at a 45° slope with the logarithm of the intensity of stimulation, we may correct for the amplifier losses and also the losses in the sound system by adding to the threshold as determined the appropriate corrections in decibels. These corrections are simply the attenuation factors of the apparatus for the frequencies in question.

Figure 5B shows the average thresholds obtained from round windows of 8 normal cats. The values are corrected for losses in both the amplifier and sound systems. Figure 5A shows the same values plotted as differences from the average thresholds of 8 normal human ears (20 to 30 yrs. of age). The cat's thresholds progressively deviate from the human as the frequency is lowered. This probably depends upon the fact that the responses to low tones are produced near the apex of the cochlea and those to high tones near the round window (Hallpike and Rawdon-Smith, 1934a; Stevens, Davis and Lurie, in press). Since we record from the membrane of the round window, the responses to low tones are more attenuated than those to high tones. The irregularities (notably those near 800 c.p.s.) are probably related to the distortion of wave form of the response due to the simultaneous presence of both action potentials and cochlear response, or possibly to a difference in acoustic impedance between cats' and human ears. These latter variations (amounting to not more than 10 db), however, are smaller than average variations in the electrical audiogram from cat to cat (17 db), so that as a first approximation these differences are not important.

The threshold curves of the action potentials in the auditory nerve follow in general the form of the thresholds from the round window. They reveal any abnormality of the nerve, such as hemorrhage in the internal auditory meatus, that may not have affected the cochlear response (Lurie, Davis and Derbyshire, 1934).

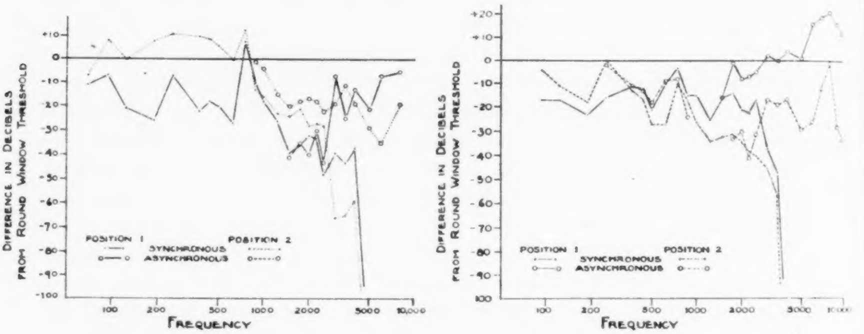
The form of the threshold curve obtained from one position in the auditory nerve differs from that obtained in another position. Figure 6 gives two examples of such pairs of positions in 2 cats. The thresholds are plotted as differences in decibels from the thresholds obtained from the round window of the same cat. These two cases are included among the 8 normal cats mentioned above.

Irrespective of the position of electrodes, the abrupt rise of threshold of the synchronized response near 1000 and the complete loss of this response between 3000 and 4000 c.p.s. are constant features of these curves. After the first rise in threshold of this response, an asynchronous discharge of nerve impulses in response to stimulation appears regularly at a lower threshold than the synchronized action potentials. Above 4000 c.p.s., the asynchronous discharge is the only response of the nerve to stimulation. The rise in threshold of both types of response between 1000 and 3000 c.p.s. is presumably the result of this division into two types of activity. Because of this division, neither one alone is powerful enough to yield a threshold as low as if there were but one type.

In both of these electrical audiograms, the threshold curves from one position of the electrode cross those from the other. In figure 6A the crossing occurs at 2500 to 3000 c.p.s., while in figure 6B it is at 360 c.p.s.

In both experiments, when the electrodes are in the dorsal part of the nerve, the curve showing greater sensitivity to the high tones is obtained. With the electrodes near the ventral portion of the nerve, the curve shows greater sensitivity to low tones.

As between these two positions, the latency of the responses to clicks differs by as much as 0.6 ms. The dorsal position, which has the shorter latency and is sensitive to high tones, reproduces spoken words clearly, although "f's" and sibilants are indistinct. The ventral position, which has the longer latency, does not yield intelligible words. In other cases



A B

Fig. 6. Audiograms of responses obtained from two positions in the auditory nerve, referred to the threshold curve from the round window of the same cat. The lower a point, the less the sensitivity of response in the nerve. The round-window threshold curve was corrected for amplifier losses (see text). The thresholds for both synchronous and asynchronous responses from each position are plotted separately.

A. (Expt. of Oct. 24, 1934.) Note loss of all synchronized response above 4000 c.p.s. This limit is the highest encountered in any animal of our series. The curves for both types of response cross between 2500 and 3000 c.p.s. The high point in the curves at 750 c.p.s. depends on an erratic point in the control (round window) threshold curve (cf. fig. 5B).

B. (Expt. of Oct. 29, 1934.) Note loss of all synchronized response above 3500 c.p.s. The curves for synchronous response cross at 360 c.p.s.

in which no difference in latency appeared with change in the position of the electrodes, there were no differences between the threshold curves from these two positions or in the reproduction of the human voice.

The variable forms of these threshold curves are not the result of a change in the character of the neural background which might differentially mask the visibility of a certain wave shape. For example, in the two illustrations given, the thresholds for low tones in the ventral position are obtained against a background of long slow waves similar to the wave shape of the response; while the dorsal position, in response to low tones, gives a sharp spike rising out of a quiet baseline. These backgrounds

favor the sensitivity from the dorsal position by yielding a clear-cut, easily discerned threshold. In spite of this situation, the ventral position is the more sensitive to low tones.

We interpret these results as indicating that particular fiber tracts convey the responses to certain ranges of tones.

Responses to impulsive stimuli. a. From the round window. It is difficult to measure accurately the latency of the electrical responses when pure tones are used as stimuli, but impulsive stimuli, i.e., "clicks," are well suited for this purpose because the wave form of each stimulus can be made extremely sharp. Such stimuli are produced by sending condenser discharges to either of the loud-speakers. The resulting sound waves from the "high" speaker are a rapidly damped series of oscillations at about 2250 c.p.s. The "low" speaker produces an asymmetric wave-train consisting of one half wave of 1000 c.p.s. and a second half wave of about 500 c.p.s. (fig. 7).

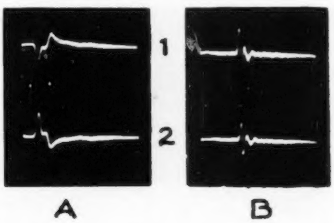


Fig. 7. Sound waves of the clicks recorded by the condenser-microphone. The time interval shown by white dots is 1 ms.

A. "Low" speaker, at −60 db: 1, normal polarity; 2, reversed polarity, showing reversal of polarity of sound waves without change of form or amplitude.

B. "High" speaker, reversed polarity: 1, at −46 db; 2, at −50 db, showing change of amplitude.

The cochlear response, as detected at the round window, does not reproduce these waves either in frequency or shape. Instead we find that the stimulation from either loud-speaker evokes an asymmetric group of electrical waves. Usually only $1\frac{1}{2}$ or 2 cycles appear at weak intensities. Their frequency is about 2000 c.p.s. when stimulated by means of the "high" speaker and about 1400 c.p.s. when stimulated by means of the "low" speaker. The second half wave is always much the larger and initiates single volleys of nerve impulses (fig. 8). Subsequent waves are present, but are visible only at 30 db or more above threshold.

At a short interval after the cochlear response, another group of waves appears. These we identify as action potentials by the four following tests: 1. They are not present after the death of the animal (fig. 8D). 2. When a scratching or hissing noise is present simultaneously with the clicks, the late group of waves is "masked," although the cochlear response remains undiminished. The phenomena of masking will be considered later in more detail (fig. 9). 3. When the electrical sign of the condenser discharge sent to the loud-speaker is reversed, the phase of negative pressure of each sound wave is reversed to a positive pressure. Likewise each phase of positive pressure becomes one of negative pressure. This change

in the sound waves produces a similar reversal of the electrical sign of the waves of the cochlear response. The later group of potentials at the round window do not reverse their polarity under these conditions but

Fig. 8

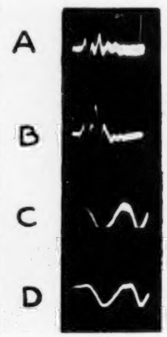


Fig. 9

Fig. 10

Fig. 8. Oscillograms of nerve impulses and cochlear response.

A. 1000 c.p.s. wave to show time scale.

B. Nerve impulses from the auditory nerve in response to single clicks from the "low" speaker.

C. Response to single clicks as recorded from round window, consisting of cochlear response followed by nerve-impulse complex.

D. The same, except for increase in strength of stimulus, after death of animal, showing persistence of cochlear response and loss of nerve impulses. No response is obtained from the nerve after death.

Fig. 9. Oscillograms from round window, showing masking.

B. Response to clicks without masking.

A. Same, masked with "hissing" sound. Note reduction of second component and constancy of first component.

D and C. Same for continuous tone of 438 c.p.s.

Fig. 10. A. 1000 c.p.s. wave.

B. Action potentials from the auditory nerve, representing nerve impulses in response to single clicks from "low" speaker.

C. Response from round window. Stimulus identical with that for B.

D. Same electrodes and strength of stimulus as B, but polarity of stimulating sound waves reversed.

E. Conditions identical with C except for reversal of polarity.

Note similarity between B and D, and similarity of second components in C and E with reversal of first component in E with respect to C.

remain, as always, negative, monophasic excursions (fig. 10). 4. Finally, the late waves always appear at least 0.6 ms. after the second half wave of the cochlear response (figs. 8 and 10). From these tests we conclude that this later group of waves recorded from the round window represents action potentials of auditory-nerve fiber.

The action-potential group is usually composed of three waves which differ in latency, maximal amplitude, and threshold. We designate these waves, arbitrarily, as *F*, *G* and *H*, in order of increasing latency. These waves are probably not related to groups of fibers possessing different diameters and exhibiting different conduction velocities. The fibers of the auditory nerve do not vary significantly in diameter, but they do differ in their distribution on the basilar membrane and in the extent of their non-medullated terminations. Experimentally we differentiate the *F*, *G* and *H* waves primarily by their latencies.

As the intensity of clicks from the "high" speaker rises from below threshold, the first waves to appear on the face of the oscillograph are *G* and *H*. At a level of 10 db above their threshold the *F* wave appears about 0.8 ms. before *G*. At this intensity the cochlear response also becomes visible about 0.8 to 0.7 ms. previous to *F*. As the stimulus is increased 20 db more, the latency of *F* decreases until its foot is within 0.6 ms. of the first negative peak of the cochlear response. This latency remains constant as the strength of stimulation is further increased. The latencies of *G* and *H* shorten like that of *F*, but precise measurements are impossible because of difficulty in identifying the feet of these later waves. The interval between *F* and *G*, measured from peak to peak, varies slightly with the polarity and the intensity of stimulation but remains consistently between 0.7 and 0.9 ms.

The *F*, *G* and *H* waves all reach their maximal amplitudes at 30 to 40 db above their own thresholds, yet with further increase in intensity of stimulation the cochlear response continues to increase.

The threshold of the cochlear response is usually higher relative to that of *G* and *H* when the clicks originate from the "high" speaker than when they are generated by the "low" speaker.

b. From eighth nerve. When the action potentials of the auditory nerve are examined by the four tests described above, they behave exactly like the waves identified as action potentials at the round window. The wave form of these potentials as recorded by coaxial electrodes may be negative or positive monophasic spikes, or else diphasic or triphasic excursions. The form depends upon at least two conditions, the direction of the bevel of the needle electrodes and the amount of the grounded needle in contact with the nerve. In these experiments the bevel is regularly parallel with the long axis of the auditory nerve and faces superiorly. The situation is complicated by the rope-like twisting of the fibers of the auditory nerve, described by Lorente de Nó (1933a), which may result in a complex electrical field inside the nerve.

The response to clicks recorded from the auditory nerve does not always show three groups of action potentials. The number found and the latency of the first group depend upon the position of the electrodes (cf. p. 486).

Velocity of conduction. The group of action potentials, either at the round window or from the auditory nerve, that is most diminished by the presence of a masking noise or tone depends upon the dominant frequency of that noise or tone (fig. 13). By virtue of this property, any particular group of action potentials may be identified as present at the round window or in the auditory nerve. When the earliest wave in the nerve is masked by the same tone or noise as *F* at the round window, the difference in latency represents a conduction time between the two points of recording. The average difference of latency in several cases was 0.14 ms. The shortest difference measured was 0.09 ms.; the longest was about 0.2 ms.

Evidence presented by Hallpike and Rawdon-Smith (1934a) indicates that the petrous bone is good insulating material. Further confirmatory evidence has been found in this laboratory by Stevens, Davis and Lurie (in press). It is reasonable therefore to assume that the action potential recorded at the round window arises from the dendritic processes of the auditory nerve lying on the basilar membrane and not from the fibers in the modiolus. The length along the nerve between the middle of the basilar membrane and the middle of the auditory nerve is 4 mm. according to measurements made by M. H. Lurie. These figures yield a conduction velocity of the order of 30 meters per second, with a possible range from 44 to 20 meters per second. These values are only approximate because of the difficulty in determining precisely the beginning of the action-potential wave of the nerve that corresponds to *F* at the round window, and some uncertainty as to the exact length of nerve fiber which the impulse has traversed.

Conduction through the cochlear nucleus involves an additional delay beyond that of conduction velocity alone. In one experiment electrodes were placed just medial to the cochlear nucleus. Here action potentials were detected in response to stimulation of the contralateral as well as the homolateral ear. The latency from stimulation of the homolateral ear was 1.0 ms. longer than that of the action potential in the auditory nerve just peripheral to the cochlear nucleus. The "synaptic" delay in the cochlear nucleus is therefore of the order of 1.0 ms.

The stimulating phase of the cochlear response. The latency of the *F* wave at the round window varies, relative to the start of the cochlear response, with both intensity and polarity of the sound waves. If stimulation of the nerve occurred at only one phase of the cochlear response, the latency of *F* measured from that phase should be constant, irrespective of polarity of stimulus. The train of sound waves produced by the clicks is an appropriate stimulus for this test, because with weak intensities the first cycle of the cochlear response dominates and is presumably the one which stimulates the nerve.

When the train of sound waves is reversed, the consequent reversal of

the cochlear response replaces its first negative peak by a positive one and the subsequent positive peak by a negative one. Therefore if the *F* wave is initiated by the first negative peak, this reversal of the cochlear response would cause a change of latency of the *F* wave. This change should be approximately equal to, and in the same direction as, the change in time of appearance of the first negative peak of the cochlear response. This would yield a constant latency of *F* from the negative peak of the dominant cycle of the cochlear response, irrespective of the polarity of the stimulus.

A typical sample of such measurements from the type of record reproduced in figure 10 is given in table 1. These results are typical of some 25 cases and show clearly that the *F* wave has a constant latency relative to the negative peak of the dominant cycle of the cochlear response. It

TABLE 1

DIRECTION OF FIRST DOMINANT PEAK OF THE COCHLEAR RESPONSE	LATENCY MEASURED FROM FIRST NEGATIVE PEAK TO FOOT OF *F*	LATENCY MEASURED FROM FIRST POSITIVE PEAK TO FOOT OF *F*
Weak stimulation		
Negative	0.92 ms.	1.14 ms.
Positive	0.88 ms.	0.76 ms.
Moderate stimulation		
Negative	0.70 ms.	1.02 ms.
Positive	0.70 ms.	0.55 ms.
Accuracy of measurement	±0.05 ms.	

is this phase that is related to stimulation of the nerve fibers. All future measurements of latency will be given relative to this phase of the cochlear response.

An interesting feature of the *F* volley is that the foot changes its latency with intensity of stimulation. This change was carefully measured in four cases and found to be approximately equal in magnitude to the duration of a half-cycle of the cochlear response. The latency is greatest at threshold and decreases until 30 db above threshold. At this level and for stronger stimulation, the latency is constant and lies between 0.5 and 0.6 ms. for all cats (fig. 11). No shorter latency than 0.5 ms. has been measured. The action potentials of the auditory nerve behave in similar fashion.

Because the change in latency with intensity is equal to a half-cycle of the cochlear response, we infer that conditions tending to excite the nerve fibers exist during this half-cycle. At threshold, this condition must act over the entire half-cycle in order to set up an impulse. At moderate and strong intensities, a brief duration is sufficient, so that the difference in

latency is one-half of a cycle. This is true within the accuracy of our measurements.

Excitation, therefore, is correlated with the phase of development of electrical positivity (i.e., negative peak to positive peak) at the round window. We have previously shown (Davis, Derbyshire, Lurie and Saul, 1934) that this corresponds to the development of negative pressure at the tympanum.

Masking. Auditory masking is a phenomenon that has been studied by means of the subjective report of an observer. It is defined as the reduction of audibility of one sound caused by the presence of another sound. We have studied the effect of such double stimulation on both cochlear response and action potentials. When a click is present simultaneously with either a hissing sound ("sss") or a musical tone, the cochlear response shows a simple summation of the electrical waves produced separately by the two stimuli. The only limitations are those imposed by non-linear distortion at high intensities. On the other hand, the action potentials in response to clicks decrease in size when the hissing sound or a tone is added. The degree of reduction of the action potentials depends upon the relative intensity of the two stimuli and is greatest when the intensity of the clicks is near threshold. Figure 9A shows the effects of a hissing sound on the response to clicks recorded from the round window and also from the auditory nerve. Figure 9C shows the effect of a noise on the responses to pure tones from the round window.

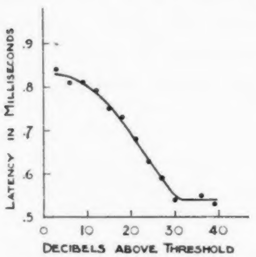


Fig. 11. (Expt. of Oct. 23, 1934.) Latency of the action potential at the round window in response to clicks from the "high" speaker, as function of the intensity of the stimulus. The latency is measured from the first negative peak of the cochlear response to the foot of the first (*F*) wave of the action potential. The total change in latency is 0.29 ms. One half-cycle of the cochlear response is 0.26 ms.

The interference of two simultaneous stimuli arriving at the ear evidently depends upon competition for the same fiber tracts. It resembles the "occlusion" of Sherrington in spinal centers. A hissing sound stimulates the nerve fibers so continuously by virtue of its several high-frequency components that the occasional stimulus from the click usually finds the nerve fibers in a refractory state. The cochlear response, which has no refractory period, can respond to both stimuli.

The masking that occurs between a tone and clicks shows two special phenomena. First, the degree of masking produced by the tone on the action potentials in response to the clicks is determined by the phase relation of click to tone. Second, the various waves of the action poten-

tial show differential masking, depending upon the frequency of the masking tone.

The effects of the phase relations are illustrated in figure 12. The greatest reduction of the click response occurs when it appears on the

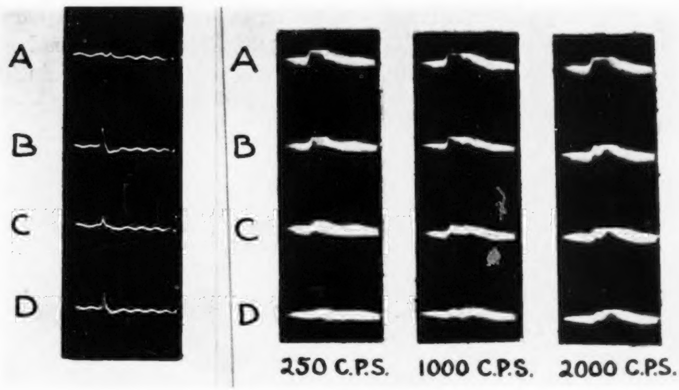


Fig. 12 Fig. 13

Fig. 12. (Expt. of Nov. 2, 1934.) Mutual interference of responses to single clicks and to continuous tone of 500 c.p.s., recorded from the auditory nerve.

A. First component of response to click almost completely masked, second component unmasked.

B. First component of click unmasked. One cycle of response to tone masked. Second component of click much reduced. Note different phase relation between tone and click as compared with A.

C and D. Intermediate phase relations with intermediate degrees of masking.

Fig. 13. (Expt. of July 18, 1934.) Oscillograms of responses from the auditory nerve to clicks at 30 db above threshold, masked by pure tones of various intensities. These are composite photographs of many responses which fall at random in various phases of the masking tone.

Masking tone of 250 c.p.s. A at 12 db above the threshold for masking. B at 18 db, C at 24 db, and D at 30 db above threshold. In A and B note the particular effectiveness on the later (*H?*) wave.

Masking tone of 1000 c.p.s. A at 6 db above the threshold for masking. B at 12 db, C at 18 db, and D at 24 db above threshold. Note greatest effectiveness on middle portion of click response.

Masking tone of 2000 c.p.s. Intensities as for the 1000-c.p.s. tone. Note selective masking in early portion of click response.

peak of the action-potential wave evoked by the tone. If the click occurs immediately before the tone, the action potential of the click is fully developed, while that of the tone is not. Careful examination of such records as these shows that the *G* and *H* waves act in the same manner, but any one of these waves may be masked independently of the others.

At the phase relation that produces the greatest masking of the *F* wave the latency between the negative peak of the cochlear response to the tone and the foot of the corresponding action-potential wave in the nerve is the same as that measured between the negative peak of the cochlear response to clicks and the resulting action potential. The latency of the latter is 0.71 ms., while the latency of the action potential in response to a 500-cycle tone is 0.76 ms. The figures agree within observational error. The agreement makes it probable that the phase of the wave coincident

TABLE 2

Responses to clicks

LATENCY OF FOOT IN MS.	THRESHOLDS IN DB	REGULARITY OF APPEARANCE	SOUNDS PRODUCING GREATEST MASKING	LATENCY OF PEAK IN MS.	GROUP
		Cochlear response			
Less than 0.1*	−90	At least 1½ cycles always present	No masking		
		Action potentials Round window			
0.5–0.6†	−85	Always	Hiss and high tones	0.7–1.0	F
	−95	Usual	"Sh" and middle tones	1.9	G
Not clearly distinguished	−95	Frequent	All low tones	2.6	H
		Auditory nerve			
0.65–0.8	−92	Groups present depend upon position of electrodes in nerve	All types, depending upon position of electrodes	0.83–1.0	

* Measured from first sound wave to foot of cochlear response.

† Measured from first negative peak of cochlear response to foot of action-potential wave.

with stimulation of the nerve endings is the same irrespective of the form of the impressed sound (i.e., click or sine wave).

The wave of the neural response to clicks which is most masked depends upon the frequency of the masking tone. The high frequencies mask the *F* waves; the low frequencies mask *G* and *H*. Figure 13 illustrates this effect. A summary of the characteristics of these responses to clicks appears in table 2.

Intensity. The relationship that the size of the cochlear response bears to the intensity of stimulation has been described previously (Davis,

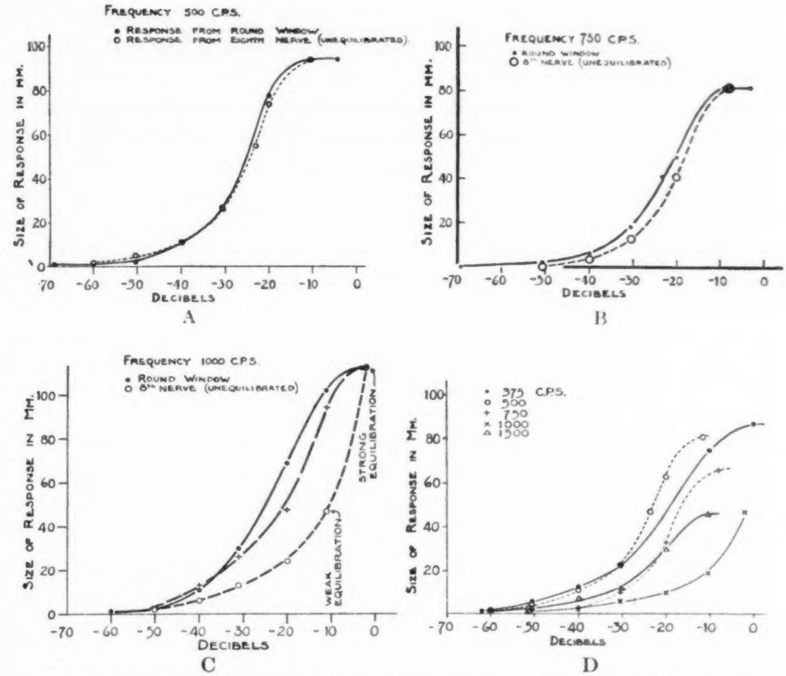


Fig. 14. Graphs representing the size of the action potential of the auditory nerve as a function of intensity of stimulation by pure tones at different frequencies and its relation to the size of the cochlear response. Zeros of intensity scales are arbitrary, representing 1 volt r.m.s. delivered to the loud-speaker. Measurements were made on the tube face of the oscillograph as quickly as possible before equilibration could occur. The relationships shown are substantiated by other experiments in which the responses were photographed and measurements made from the film.

A. Frequency 500 c.p.s. Heavy line, response from round window; dotted line, response from auditory nerve (unequilibrated). The actual measurements of the response of the auditory nerve have been multiplied by a factor such as to make the maximal response coincide with the maximal response measured from the round window.

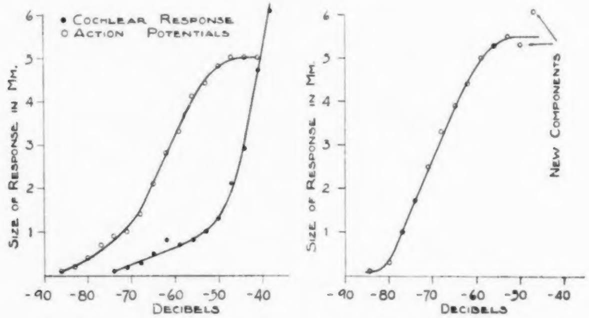
B. Frequency 750 c.p.s.; otherwise as in A.

C. Frequency 1000 c.p.s. The dotted line represents the actual measurements of the responses of the auditory nerve multiplied by a transformation factor as in A and B. With maximal stimulation, however, there was very marked and rapid equilibration, which was absent with weaker stimulation. As explained in the text this probably indicates alternation of response with weak stimulation and no alternation with maximal stimulation. The values for weak stimulation are therefore multiplied by 2 and are represented by plus sign and broken line in the graph.

D. The complete family of curves of this same experiment, all obtained from the same auditory nerve at different frequencies. For a comparable study of the cochlear response, see Davis, Derbyshire, Lurie and Saul (1934, fig. 8, p. 321).

Derbyshire, Lurie and Saul, 1934). The size of the action potential of the auditory nerve follows closely the same relationship if the measurements are made before equilibration occurs.

Figures 14A, B and C show the measurement of the height of the action potential after the "on effect" in response to different intensities of stimulation at 500, 750 and 1000 c.p.s., respectively. These measurements are made directly on the tube face of the cathode ray oscillograph as soon after the beginning of stimulation as possible. In all three figures the size of the maximal action potential is arbitrarily made equal to the maximal cochlear response at the same frequency and from the same animal. Figure 14D shows an entire family of these curves.



A B

Fig. 15. (Expt. of Oct. 23, 1934.) The cochlear response and the action potentials in response to clicks from the "high" speaker, as function of intensity of the stimulus.

A. The cochlear response and the action potential from the round window.

B. Action potential from the auditory nerve.

At −60 db, the latency of the action potential at the round window is 0.64 ms., while in the auditory nerve it is 1.64 ms. This great difference in latency suggests that in this case we are not dealing with the same nerve fibers. This is corroborated by the fact that the two intensity curves for the action potentials do not superimpose.

Figure 14C shows a special case of the amplitude of the action potentials as a function of intensity. At this frequency, 1000 c.p.s., the nerve fibers are going into alternation. At low intensities there is little equilibration, while at maximal stimulation it is marked. Below maximal, therefore, the nerve fibers are already in alternation when measured. At maximal, however, they still respond to every sound wave at the time of measurement because of the strong stimulation. The values obtained below maximal should therefore be doubled to allow for this, and a more satisfactory correspondence results. Near 1000 c.p.s. the discrepancies between the intensity curves of the action potentials and of the cochlear response are usually greatest. This is to be expected as a result of the irregularities introduced by alternation.

To avoid these complications we may study the size of the action potentials at the round window as a function of intensity of stimulation by clicks. Even so, we encounter the difficulties that, if too strong a stimulus is used, the later waves of the cochlear response become significant and distort the superimposed action potentials, and also that each action-potential wave must be separately considered. The results with moderate stimuli are shown in figure 15.

DISCUSSION. *Properties of auditory nerve fibers.* Lorente de Nó (1933b) measured the diameter of the fibers of the auditory nerve as 5μ. According to the generalizations of Blair and Erlanger (1933), this value is consistent with our estimation of the conduction velocity as 30 meters per second and of the refractory period as 1.0 ms. These fibers are evidently similar to the medullated sensory fibers in peripheral nerves. There is certainly no evidence of unique properties, such as the unusually brief refractory period postulated by Boring (1926) and Troland (1929).

Our value of 1.0 ms. for the refractory period of these fibers was obtained indirectly through the determination of the frequency at which alternation of activity appeared. This method measures the refractory period of the slowest part of the nerve fiber which the impulse must traverse. The refractory period of the non-medullated termination of these fibers in the organ of Corti is therefore not more than 1.0 ms. Lorente de Nó (1935) has recently made a somewhat similar measurement of the refractory period of the motoneurons serving the external rectus muscle of the eye. He found that the longest absolutely refractory period in the path from the axon of the internuncial neuron through its terminal twigs to the cell body and axon of the motoneuron was 0.6 ms. His result and ours suggest that the refractory periods of small twigs, whether of axons or dendrites, do not depend upon their diameter or degree of myelination and are no longer than the refractory period of the myelinated portion of the neuron to which they belong.

Origin of cochlear response. Theories of the origin of the cochlear response are of two types: 1, the non-neural hypothesis, such as that advanced by Davis, Derbyshire, Lurie and Saul (1934) which relates the cochlear response to activity of the sensory hair-cells, and 2, the neural theory proposed by Hallpike and Rawdon-Smith (1934c). The latter theory was advanced to explain their observations on one cat in which the auditory nerve had been severed, with conservation of the blood supply to the cochlea, and the neural elements allowed to degenerate. This animal yielded no cochlear response, but all structures except the nerve fibers and ganglion cells appeared normal under the microscope. They tentatively interpreted the cochlear response as the action potentials of the terminal branches of the nerve fibers in the organ of Corti. We believe that this is impossible in view of our evidence of fundamental differences between the cochlear response and the neural response. These

differences include the relative resistance of the cochlear response to anemia, the reversibility of polarity shown by the cochlear response but not by the action potentials, the different limits of frequency, the fatigue, equilibration and susceptibility to masking of the neural response, and, finally, the latency of the neural response, which places it always at least 0.5 ms. after the cochlear response.

The case reported by Hallpike and Rawdon-Smith differs from those of Guttman and Barrera (1934), who found the cochlear response present "at all intervals tested, from 10 days to 6 weeks" following transection of the eighth nerve. We tentatively ascribe its absence in Hallpike and Rawdon-Smith's case, 6 months after transection, to the onset of degenerative changes in the denervated sensory cells, in spite of their normal morphological appearance, and we adhere with confidence to our view that the cochlear response is generated by the sensory cells of the organ of Corti.

Mechanism of excitation of the auditory nerve. The question now arises as to what part, if any, is played by the cochlear response in the mechanism of hearing. Davis, Derbyshire, Lurie and Saul (1934) suggested that these potential changes might serve to stimulate the nerve endings. This concept is difficult to reconcile with our present measurements of the latency of the action potentials at the round window. This delay of 0.5 to 0.6 ms. may be explained by one or more of the following possibilities: 1, slow conduction in the non-medullated terminations of the nerve endings; 2, a delay of conduction through the ganglion cells in the spiral ganglion; 3, the utilization time of the stimulus acting on the nerve fibers; 4, a delay in liberation and action of a chemical mediator.

In regard to the first possibility, it is doubtful whether there is a long enough section of non-medullated nerve to account for this delay. Lorente de Nó (1933b) showed that the internal hair-cells and probably some of the external hair-cells are innervated by very short sections of non-medullated nerve, usually not more than 30μ in length. It is true that the external spiral fibers may pass "basalward" as non-medullated strands for as much as a third of a turn along the basilar membrane; but, if at the round window we are able to detect impulses set up by the internal hair-cells, the non-medullated section is certainly not long enough to explain a minimum delay of 0.55 ms. This would require a velocity of conduction of about 6 cm. per second. The following considerations indicate that we detect at the round window the nerve impulses initiated by the internal hair-cells. In all of our explorations of the eighth nerve, we have never found an action-potential wave in response to clicks which appeared earlier than the F wave at the round window. This is true even when the intensity of stimulation is practically maximal. In five experiments, the nerve was systematically explored with coaxial electrodes with special attention to this point. If impulses earlier than the F wave

were set up by the internal hair-cells and escaped detection at the round window, they should certainly have been found in one or another of these explorations of the nerve. We therefore conclude that the impulses initiated by the internal hair-cells have a latency at least as great as that of the *F* wave. It is unreasonable to explain this latency on the basis of slow conduction over a non-medullated twig as short as 30μ.

It is conceivable that the action potentials recorded from the round window are generated by the cell bodies of the spiral ganglion or by their axons and that for some reason we are unable to record the action potentials of the dendrites. We might then assume a delay in the transmission of the impulse across the cell body (Erlanger, Bishop and Gasser, 1926). However, it is unlikely that we could record action potentials readily from within the modiolus, where the active nerve is separated from the round window by a layer of bone, and not at all from the terminal fibers which lie in a comparatively exposed position on the basilar membrane. Consequently, both of the above assumptions seem most unlikely and quite gratuitous, and may be discarded until more direct evidence in favor of them is available.

The third possibility is a long utilization time for stimulation by the cochlear response; but we have shown by measurement of the frequency at which alternation occurs that these non-medullated nerve endings have a refractory period of not more than 1.0 ms., and, on this basis, we should expect a utilization time similar to medullated fibers. The utilization time of a medullated fiber in a frog's nerve is much less than 0.1 ms. for strong stimuli. This is certainly too short to account for the experimentally found delay of 0.55 ms. in a warm-blooded preparation. If a utilization time of 0.5 ms. is required for strong stimulation, it is very difficult to see how an individual fiber could respond to each sound wave of a 1000-cycle tone of moderate intensity, for with such a tone the stimulating phase lasts only 0.5 ms.

The situation is precisely like that of synaptic or neuromyal delay in which the duration is of the same order of magnitude, and for which the same possibilities of slow conduction in the terminal twigs and long utilization time have been advanced in explanation. In few cases can these arguments be disposed of as completely as in the present one. Apparently we are dealing with the general case in which one cell excites another, and we find difficulty explaining it by the same mechanism which seems to account for conduction within a single neuron. We must consider seriously the possibility of a chemical mediator liberated by the sensory cell, at the synapse, and at the neuromyal junction. Chemical mediation would at least account for the delay and in our special case would overcome another difficulty faced by the theory of electrical stimulation, namely, the problem presented by the electrical polarity of the sensory cell during the phase of stimulation and the anatomical relationship be-

tween the cell and the basket-work of terminal twigs of the nerve around its base and sides (Kohlmer, 1927). We have shown that stimulation occurs while the round window is becoming electrically positive and we have previously shown (Davis, Derbyshire, Lurie and Saul, 1934) that the electrical polarity of the base of the hair-cell corresponds to that which is recorded at the round window. Apparently, therefore, the part of the hair-cell in contact with the terminal nerve twigs is becoming electrically positive at the moment of stimulation. This is contrary to any simple expectation in terms of the laws of electrical excitation, and requires subsidiary hypotheses for explanation. The difficulty does not arise with the theory of chemical mediation.

Interpretation of F, G and H waves. The *G* and *H* waves of response at the round window appear 0.9 ms. or more after the *F* wave. This extra delay may perhaps be interpreted as the result of conduction through a greater length of nerve than is required for the *F* impulses. We are still unable to identify these waves with any particular anatomical groups of nerve fibers. It is tempting to think of the *F* wave as representing the internal fibers and the *G* and *H* the direct and spiral external fibers, respectively, but this does not explain their different behavior toward masking tones of different frequencies. It may equally well be that the *F* wave is earliest because set up nearest the round window by the incoming pressure wave. Further experimentation is necessary before we can settle this point, and we wish to emphasize the fact that these waves are characteristic of a particular mode of stimulation, by clicks, and may represent a special case not to be taken too seriously in forming generalizations.

Psychophysiological considerations bearing on the theory of hearing. Our determination of the limiting frequency of response of the individual nerve fibers at 1000 c.p.s. and the appearance of alternation of response at higher frequencies, with the loss of synchronization of any action potentials above 4000 c.p.s., show definitely that the sensation of pitch is not determined for high tones by the frequency of impulses in the fibers of the auditory nerve. We have developed these considerations elsewhere (Davis, Derbyshire and Lurie, 1934). Presumably the mechanism of pitch perception is similar for all parts of the audible range and is independent of the frequency of nerve impulses at the low, as well as at the high, frequencies. This leads us necessarily to some form of resonance or "place" theory along the lines originally suggested by Helmholtz, whereby pitch is correlated with the particular group of neurons activated. Our observation that certain regions in the auditory nerve yield responses to faint tones of low frequency and are unresponsive to equally intense high tones, while another region shows the reverse relationship, is strong support for this type of theory.

Auditory fatigue in man was studied by v. Békésy in 1929. It is of

interest to examine his data to see whether they reflect the process of equilibration of the auditory nerve impulses and the particular relation which equilibration bears to frequency. He found a maximum reduction of acuity to a fatiguing tone after 2 minutes of stimulation and full recovery of acuity after 15 seconds. There is some individual variation in the degree of loss, but for any one observer the rate and degree of loss are constant for all frequencies between 300 and 2000 c.p.s. There is no suggestion of any auditory experience that correlates with the particularly rapid and extensive equilibration which occurs in the auditory nerve of the cat at frequencies near 1000 and 2000 c.p.s. When we listen to such tones, the individual nerve fibers must soon fall into alternation of response, but we are not aware of any subjective change of either pitch or loudness. Auditory fatigue might seem at first glance to correspond to the phenomena of equilibration, but it apparently fails to show the critical frequency bands at which we should expect to find it most strongly marked. If subjective pitch is not determined by frequency of nerve impulses but by the region of resonance of the basilar membrane, the constancy of pitch during equilibration is not surprising. We are now forced to conclude further that the frequency of impulses in each fiber is not the basis of the sensation of loudness. Hearing is like the cutaneous-tactile sense, which perceives increasing frequency of contact as vibration and not as greater intensity of pressure, and it differs from muscle sense and the sense of pressure, in which the frequency of discharge of the sense organs is one determinant of the intensity of our sensations (Adrian, 1928).

The problem of the physiological correlate of loudness still remains. Some of our data serve to illuminate it in part. In the first place, the threshold of the cochlear response in cats agrees closely with the auditory threshold of man, and, what is more important, this correlation apparently extends to the threshold for nerve impulses in the auditory nerve if slight allowances are made for the relative difficulty of detection of the nerve impulses. We have already shown that the amplitude of the cochlear response bears a relationship to the logarithm of the intensity of the stimulus, which, through the range of medium intensities, agrees with the classical Weber-Fechner law. We now find that the amplitude of the recorded action potentials, measured before equilibration, correlates closely with the amplitude of the cochlear response. Because the frequency of nerve impulses in each fiber is fixed by the frequency of the stimulus (at least below 700 c.p.s.), a change in intensity of stimulation at such a frequency can increase the amplitude of the total action potential only by increasing the number of active neurons, since the individual nerve impulses obey the all-or-none law. Near 1000 c.p.s. and above, the same relationship holds as far as the synchronized impulses are concerned, although it is complicated by alternation. As yét, we are unable

to make quantitative measurements of the asynchronous impulses, but we may fairly assume, on the basis of consistency and simplicity, that the relationship of the number of active fibers to the intensity of stimulation is the same. We infer that the number of active neurons in the auditory nerve correlates closely with the amplitude of the cochlear response as we vary the intensity of stimulation. At present, these seem to be the most probable physiological correlates of the sensation of loudness in the sense organ and in the afferent nerve, but more precise and direct comparisons are needed before we may consider this generalization adequately established.

We do not see in either pitch or loudness any auditory phenomenon which seems to be determined by the quantal nature of nerve impulses or other limitations of the nervous mechanism. For masking, however, the case is different. This seems to depend clearly on the existence of an "all-or-none" type of response and a refractory period. Because of these characteristics of the nerve impulse, simultaneous stimuli of a repetitive sort, such as sound waves, must compete for the particular nerve fibers. If the fiber has responded to one stimulus, it cannot, within a certain length of time, respond to the other. There is in this no summation, but exclusion. This "line-busy" effect is at least one of the physiological mechanisms responsible for masking and is imposed by the auditory nerve and not by the sense organ.

SUMMARY

The action potentials of the auditory nerve of cats in response to stimulation by sound have been led off by coaxial electrodes and studied by means of a cathode ray oscillograph. They have been compared with the electrical responses of the cochlea recorded from the round window.

During continued stimulation, the action potentials of the nerve show a progressive diminution in size (equilibration) which is a function of the frequency of stimulation as in other medullated nerves. There is a "fast" equilibration complete within 2 seconds or less, which depends primarily on the development of alternation of response (p. 480), i.e., each nerve fiber responds only to alternate sound waves. There is in addition the familiar "slow" equilibration, which is complete in about 7 minutes. Recovery requires about 30 seconds. There is no equilibration of the cochlear response.

Above a critical frequency of the stimulating sound, 1000 c.p.s., the size of the unequilibrated response is about half that below 1000 c.p.s. (fig. 2). A second similar critical frequency, an octave higher, is usually apparent. At a frequency of 3000 or, at most, 4000 c.p.s., the nerve impulses are no longer synchronized with the sound waves, but form a completely asynchronous discharge. The lowest critical frequency indicates alternation of activity and yields a measure of the "functional"

refractory period of the sensori-neural mechanism, including the non-medullated peripheral terminations of the axons. In good preparations, this is not greater than 1 millisecond, and the maximal frequency of impulses in each nerve fiber is 1000 per second.

The threshold of cochlear response for normal cats is practically identical with the auditory acuity of normal human observers (p. 485). The threshold of response of the auditory nerve at various frequencies depends on the particular group of fibers within the nerve which make contact with the coaxial recording electrodes. The greatest sensitivity is practically the same as for the cochlear response. This localization within the nerve of sensitivity to particular tones supports the "place-resonance" theory of audition.

The response to impulsive stimuli (clicks) recorded at the round window consists of the cochlear response, followed by one to three groups of action-potential waves (p. 489). The earliest of these has a latency of at least 0.6 ms. with respect to the first negative peak of the cochlear response. Identification of these groups of action potentials in the auditory nerve is uncertain, since their presence or absence depends in part on the exact position of the recording electrodes.

The velocity of conduction in the fibers of the auditory nerve is approximately 30 meters per second (p. 490).

The nerve impulses are initiated during the phase of the cochlear response in which the round window is becoming electrically more positive (p. 490). This corresponds to the development of negative pressure at the tympanum. Excitation occurs earlier in this phase with strong stimulation than it does with weak stimulation (fig. 11).

The response of the nerve to a noise or pure tone may be masked by simultaneous stimulation with another noise or tone (p. 492). Masking does not occur in the cochlear response, but instead there is algebraic summation. Masking in the nerve depends on the refractory period of the nerve fibers. The earliest action-potential wave in the response to a click is masked most effectively by high tones, and the later potential waves, by medium and low tones.

At frequencies low enough to avoid alternation of response and before equilibration occurs, the amplitude of the action potential of the nerve bears the same relation to the intensity of the stimulating sound as does the amplitude of the cochlear response (fig. 14).

The numerous differences between the action potentials of the auditory nerve and the cochlear response show conclusively that the latter is non-neural in character.

Various possible explanations for the delay between the cochlear response and the corresponding action potential in the nerve are considered. We believe that it is unreasonable to attribute this delay solely to conduction time and suggest that it depends upon a process involving chemi-

cal mediation of excitation between the sensory cell and the nerve fiber (p. 498).

The above evidence strongly favors a "place-resonance" theory of pitch perception. No subjective aspect of hearing correlates with the phenomena of alternation of response of the nerve fibers or equilibration in the size of the action potentials. Loudness probably correlates with the total number of active fibers rather than with any simple function of the number or frequency of nerve impulses. Masking is the only limitation of hearing which can clearly be attributed to the neural mechanisms of hearing thus far investigated.

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